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(57) Abstract

Calixarene-based compounds are described which have biological activity, particularly anti-bacterial, anti-fungal, anti-cancer and anti-viral activity. Some compounds have been found to have anti-HIV activity. The compounds are calixarenes or oxacalixarenes, acylic phenyl-formaldehyde oligomers, cyclotriveratrylene derivatives, cyclic tetrameric resorcinol-aldehyde derivatives known as Hogberg compounds and cyclic tetrameric pyrogallol-aldehyde derivatives.

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CALIXARENE-BASED COMPOUNDS HAVING ANTIBACTERIAL, ANTIFUNGAL, ANTICANCER HIV ACTIVITY

The present invention relates to compounds having biological activity, particularly calixarene-based compounds, having anti- bacterial, anti-fungal, anti-cancer and anti-viral, particularly anti-HIV activity.

The virus that causes AIDS, the human immunodeficiency virus HIV is believed to be one of the major threats to human life and health worldwide. Even back in 1988 an article in Scientific American by J. M. Mann, J. Chin, P. Piot and T. Quinn estimated that more than a quarter of a million AIDS cases had occurred up to then and that 5-10 million people were infected with HIV worldwide.

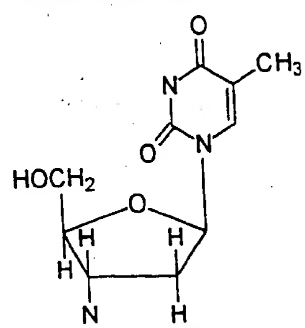
The HIV has been studied more intensively than any other virus and we now have a general picture of how the genes and proteins in the HIV virus particle operate, although we don't have a clear understanding of what controls the replication and how it destroys the human immune system. There are in fact many strains of HIV. The two main ones are HIV-1 and HIV-2. HIV-2 is prevalent in West Africa and produces a less severe disease than does HIV-1 the most common form elsewhere.

The life cycle of the virus is described below in some detail since for a drug to be effective it has to interfere with at least one stage of its life cycle. The HIV virus particle is roughly spherically shaped and is about a thousandth of a millimetre across. Its outer membrane consists of lipid molecules which possess many viral protein spikes projecting outwards. Each spike is thought to consist of four molecules of glycoprotein gp120 with the same number of glycoprotein gp41 molecules embedded in the membrane itself. These envelope proteins come into play when HIV binds and then enters target cells. Gp120 can bind tightly to CD4 proteins sited in the membranes of immune system cells especially T lymphocytes also called T cells. This is the first stage of the infection which is followed by fusion of the virus and T cell membrane, a process governed by the gp41 envelope protein. The result is that the contents of the virus core are thus freed to enter the cell. The virus core is surrounded by matrix protein called p17 and is itself in the shape of a hollow cone made of another protein p24 containing the genetic material of the virus.

Being a retrovirus this genetic material is in the form of RNA

(ribonucleic acid) consisting of two RNA strands. These are in turn attached to molecules of an enzyme, reverse transcriptase, which transcribes the viral RNA into DNA once virus has entered the cell. Coexisting with RNA are an integrase, a protease, a ribonuclease and other enzymes. Once in the cell the viral RNA is converted to DNA which then enters the cell nucleus. The next step is integration of viral DNA into host chromosomes. This is followed by cell proteins binding to DNA initiating transcription. Short RNA molecules then leave the nucleus and make viral regulatory proteins followed by medium length and long RNA which generate structural and enzymatic proteins. These assemble to form new viruses (replication-viral budding) (1).

The drug of choice in AIDS treatment up to this time has been Wellcome's Retrovir or Zidovudine which is 3'-Azido-2',3'- dideoxythymidine or AZT for short:



This compound is of the dideoxynucleoside type and blocks HIV replication by inhibiting reverse transcriptase. (Such compounds are actually modified _in vivo_ in the target cell to active 5'-triphosphates (2). Other nucleoside drugs believed to work in a similar way are Didanosine ddl (or Videx) which has been developed by Bristol-Myers Squibb and dideoxycytidine ddC of Roche. 2',3'-Didehydro-2',3'- dideoxythymidine Stauvudine, D4T was developed by Bristol-Myers Squibb after ddl as a cheaper alternative to AZT (2,3).

However, the important point to be made here is that all these drugs are highly toxic, potentially nerve damaging materials. In addition AZT can give rise to anaemia although this undesirable side effect may be counteracted by co-administration of drugs such as erythropoietin. The least toxic alternative to AZT is Triton Bioscience's 3'-azido-dideoxy-uridine AzdU but being structurally similar to AZT may have similar resistance problems (3). Indeed apart from the toxic side effects associated with the use of AZT the virus quickly develops resistance to this drug (4). Researchers had hoped for several years that using a combination of these nucleoside inhibitors would provide benefits over individual drugs used alone. However, recent such results presented in Berlin were very disappointing. A large recent

controversial French and British Study of AZT indicates that its early use in HIV-infected individuals provided no survival benefits (5).

New non-nucleoside reverse transcriptase inhibitors which have selective anti HIV-1 activity are certain benzodiazepine analogs and thione derivatives developed by Pauwels and coworkers (2) but again resistance to these compounds develops very rapidly blunting their clinical usefulness.

Several new drugs have found to help block the step prior to that involving reverse transcriptase i.e. the transcription of RNA to double stranded DNA which is the step of entry, uncoating and RNA release. These are bicyclams and hypericin currently undergoing clinical trials (2). Another approach targetting the even earlier step, that of the binding of gp120 to CD4 has involved utilisation of soluble CD4 to flood the body and act as a decoy for the virus or attachment of CD4 to an antibody or antibody-toxin complex. However, again results have been very disappointing.

Low molecular weight dextran sulphate has been demonstrated to block the binding of the HIV virus particles to CD4 (its target cells) in *in vitro* testing. However, again clinical testing provided no benefits, probably related to the ease of degradation of this anionic polysaccharide (6).

A wide range of known and potential anti HIV-1 agents were tested for their *in vitro* anti HIV-1 activity (7). Apart from AZT the most active agent found was RO 31-8959 (XVII) a compound developed by N. A. Roberts and coworkers (8). This compound worked at a much later stage in the life cycle of the HIV virus namely as a HIV-1 protease inhibitor. In fact two protease inhibitors have now entered clinical trials (2).

More recent developments have involved the use of anti-sense oligodeoxynucleosides (short segments of DNA) that may hybridize to messenger RNA and inhibit translation. In any event they have been demonstrated to possess *in vitro* anti HIV activity (2).

A very late stage in the HIV life cycle which has been targetted is that of viral budding which has at least been partially blocked by use of interferon alpha.

Prem Mohan and coworkers of the University of Illinois, Chicago have

recently improved the anti HIV activity of naphthalene disulphonic acid derivatives to 6 μM for HIV inhibition (4) in <u>in vitro</u> testing. He believes they work by acting at the earliest stage of the HIV virus life cycle namely binding onto gp120 on the virus's surface. He believes that certain sites on the HIV's protein carry positive charges and that the negatively charged sulphonic acids can block these and prevent the virus entering its target cell. Mohan's coworker Sandeep Varma has recently found that these molecules <u>also</u> inhibit reverse transcriptase.

Another very recent development reported is utilisation of soluble fullerenes to inhibit the key viral enzyme HIV-protease competitively at 5 μM concentration by S. H. Friedman and coworkers at UCSF (9) in <u>in vitro</u> testing.

However, the levels at which these novel agents are effective is relatively high and little is known of their toxicity to healthy cells (cytoxicity).

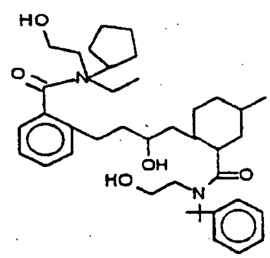
The concentration at which an HIV-1 drug is effective is designated EC_{50} which represents when the number of cells protected from HIV injection is half of the total. The antigen Agp120 assay - the virus related antigen - is related to the number of virus particles produced by measuring glycoprotein gp 120 in infected cell cultures uM (micromolar). Thus EC_{50} represents the concentration which reduces required Antigen gp 120 by 50% in infected cell cultures. The concentration of the drug which reduces cell growth by 50% is designated TC_{50} μM.

Of course the lower the EC_{50} concentration the better but the real criterion of effectiveness in <u>in vitro</u> testing on cell cultures is the Therapeutic index which is TC_{50}/EC_{50} ratio so as not to damage healthy cells. Thus AZT has an EC_{50} of <u>Ca</u> 0.016 μM with a TC_{50}>1000 uM. This results in a therapeutic index of >1000/0.016 = > 62,500. Of course human beings and animals are more than a collection of cells and in spite of the high Therapeutic Index, AZT is quite toxic, giving rise to nerve damage and anaemia among other things (3). Nevertheless such tests on cell cultures indicate what is a potential anti HIV drug.

Other factors relevant to the usefulness of an anti HIV drug are physical properties such as water-solubility for drug absorbtion by the patient and stability of the compound after oral intake. Thus the potentially useful

drug, the anionic polysaccharide, dextran sulphate is poorly absorbed orally and degrades after oral intake before entry into the plasma (6). Another important factor is ease of synthesis of the drug and hence drug cost which is relatively high for AZT and most other drugs produced to date which are potentially useful in combatting AIDS.

Very recently Agowan Pharmaceuticals, San Diego have developed orally active compounds that are potent inhibitors of a key enzyme of HIV namely HIV protease. The compound has been called AS-1284(10)



It is an object of the present invention to provide novel, and easily synthesised compounds having biological activity, particularly having improved anti-HIV activity.

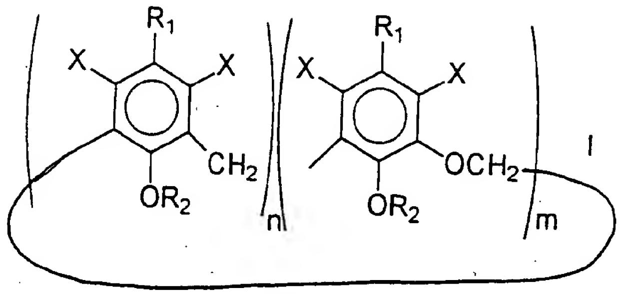
Such compounds are cyclic and acyclic phenol-aldehyde oligomers and their wide range of derivatives preferably carboxylic acid salt derivatives which renders them water soluble. These compounds have shown surprisingly good anti-HIV activity, particularly against HIV-1.

The first class of compounds are derived from cyclic phenol-formaldehyde calixarenes and oxacalixarenes.

Polyoxyethylene ethers of calixarenes have been shown to have biological and biochemical effects (Jain et al Biochem J. (1985), 227 p. 789-94). At least some of the derivatives of calixarenes/oxacalixarenes of the present invention possess metal ion complexing ability. A range of these already known to complex metal ions has been described:- US Patents 5,132,345; 4,556,700; 4,642,362; 4,866,198; 4,882,449; 4,699,966; 4,855,461; 4,908,399; 4,933,407; EP 237,265; 262,910 and 309,291.

The present invention provides calixarene or oxacalixarene derivatives of

the formula I



wherein $n + m = 3 - 8$
$m = 0 - 3$
$n = 0 - 8$

R^1 is H, halogen, hydrocarbyl, aryl, hydrocarbylaryl or a substituted derivative thereof, NO_2, SO_3M where M is an alkali metal, SO_3H, $R^1 = OR^2$, R^2 described below,

X is halogen, NO_2, CO_2H, CN or other electron withdrawing group.

$$R^2 = CH_2\overset{O}{\overset{\|}{C}}OR^3 \quad \text{or} \quad CH_2\overset{O}{\overset{\|}{C}}O\underline{M^P}_{P} \quad \text{or} \quad CH_2\overset{O}{\overset{\|}{C}}N\begin{matrix} R^4 \\ R^5 \end{matrix}$$

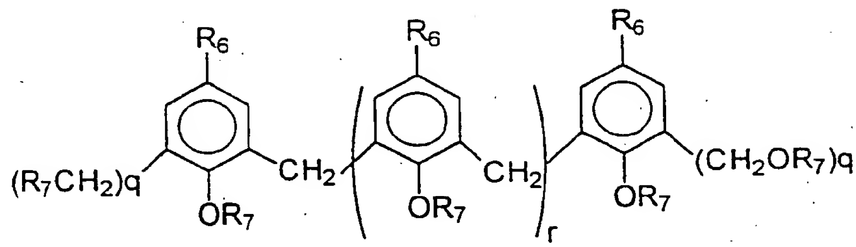
R^3 is alkyl or a substituted derivative thereof,
M is a metal or ammonium ion, P is the charge on the metal ion, R^4 or R^5 may be the same or different, or both may be part of amino acid ester or poly(amino acid ester) of one or more of the same or different amino acids or part of a cyclic polyene antibiotic/antifungal drug or part of a cyclic nitrogen heterocycle,
R^1 is preferably NO_2 or a halogen, particularly bromine, R^3 is preferably $CH_2CH_2OCH_3$ when R^1 is ethyl, n is 7 and m is 0, M is preferably an alkali metal or alkaline earth metal or ammonium or a substituted derivative thereof, R^2 is preferably CH_2CO_2K or $CH_2CO_2NH_4$.

The cyclic polyene drug may be Amphotericin B or a lactam antibiotic such as a penicillin derivative or the aminoglucoside sinefungin.

The cyclic nitrogen heterocycle may be an aminotetrazole or aminotriazole.

In a second aspect the invention provides open chain, i.e. acyclic

phenol-formaldehyde oligomers of formula II



II

wherein q = 0-1, r = 0-6; R^6 is alkyl, H, halogen, aryl, alkaryl or a substituted derivative thereof,

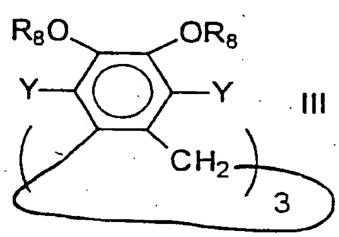
R^7 is H or $CH_2CO_2\underline{M}^p_p$ where p is the charge on the metal ion,

M is a metal ion,

R^6 is preferably halogen. M is preferably an alkaline metal or alkaline earth metal. R^7 is preferably CH_2CO_2K.

Phenolic oligomers may be made by the procedure of B. Dhawan and C.D. Gutsche, J. Org. Chem 1983 48 p.1536, and the ester modified oligomers by the procedure of U.K. Pat. Appln. GB 2,200,124 A1 by S.J. Harris and B.J. Kneafsey assigned to Loctite (Ireland).

In a third aspect the invention provides cyclotriveratrylene derivatives of formula III



III

wherein Y is H, halogen, NO_2

R^8 is H or $CH_2CO_2R^9$ or $CH_2CO_2\underline{M}^p_p$

R^9 is alkyl, aryl, alkaryl or a substituted derivative thereof,

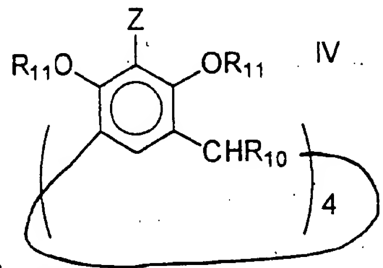
M is metal ion, and p is the charge on the metal ion.

Preferably Y is halogen and R^8 is CH_2CO_2K.

M is preferably an alkaline metal or alkaline earth metal.

The parent cyclotriveratrylene may be synthesised by the process of J.Org. Chem 43 (9) 1978 p.1808 by J.A. Hyatt.

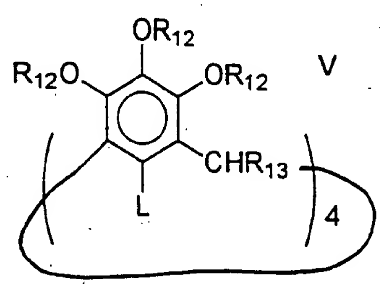
In a fourth aspect the invention provides cyclic tetrameric resorcinol-aldehyde derivatives known as Hogberg Compounds, (J.Org. Chem. 1980 45 p.4498 by A.G.S. Hogberg) of formula IV



IV

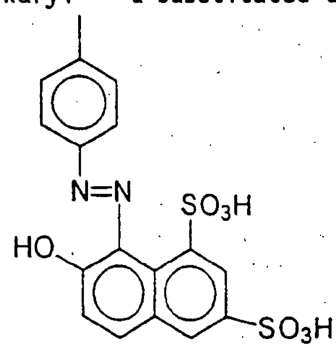
wherein R^{11} is the same as R^8 defined above, Z is halogen or nitro, R^{10} is alkyl, aryl, alkaryl or a substituted derivative thereof. Preferably Z is halogen and R^{11} is CH_2CO_2K.

In a fifth aspect the invention provides cyclic tetrameric pyrogallol-aldehyde derivatives, (Eur. Pat. Appl. EP 400,773 5th Dec. 1990 by J. Holmes, P.Tasker of ICI) of formula V

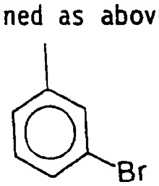


V

wherein R^{12} is the same as R^8 defined above,
L is H, halogen or nitro, or other electron withdrawing group,
R^{13} is alkyl, aryl, alkaryl or a substituted derivative thereof or when R^{12}=H, L=H, R^{13}=

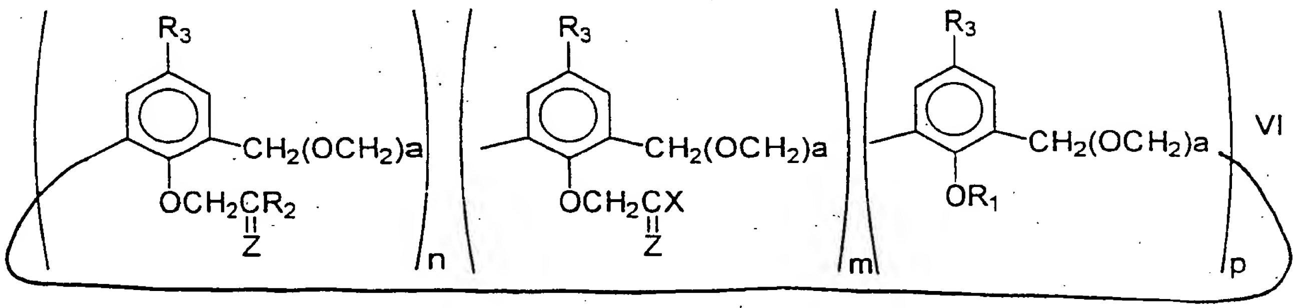


Preferably L is halogen e.g. Bromine, R^{12} is $CH_2CO_2NH_4$, CH_2CO_2K or CH_2CO_2M where M is defined as above, and R^{13} is preferably $(CH_2)_2CH_3$ or



These are the most active compounds with similar activity to AZT and very high TC_{50}/EC_{50} therapeutic indices with the added benefit of the metal acetates being <u>very</u> soluble in water.

The present invention provides in a sixth aspect calixarene or oxacalixarene derivatives of the formula VI



VI

wherein

n + m + p = 3-8

m = 0-8

n = 0-8

p = 0-8

a, which may be the same or different on each aryl group, is 0 or 1;

R', which may be the same or different on each aryl group, is -H or hydrocarbyl, aminohydrocarbylaryl, hydrocarbylaryl or a substituted derivative or salt thereof;

R^2, which may be the same or different on each aryl group, is hydrocarbyl, aryl, hydrocarbylaryl, hydrocarbyloxy, aryloxy, hydrocarbylaryloxy, alicyclic, hydrocarbylthio, arylithio, hydrocarbylarylthio, oxime, or a substituted derivative thereof;

or $-N(R^4)(R^5)$

wherein R^4 and R^5, which may be the same or different, are -H or hydrocarbyl, aryl, hydrocarbylaryl or a substituted derivative thereof and R^4 and R^5 may form a cycloaliphatic ring, which may in turn be substituted; or R^5 may be

$$-\mathrm{N}\begin{array}{l}\diagup R^6\\ \diagdown R^7\end{array}$$

wherein R^6 and R^7, which may be the same or different are H or hydrocarbyl, aryl, hydrocarbylaryl or a substituted derivative thereof; or R^5 is OR^4 wherein R^4 is as defined above; or R^5 is a residue of a hydrocarbyl, aryl or hydrocarbylaryl group or of a substituted derivative thereof providing bond to another calixarene or oxacalixarene derivative wherein R^5 is a similar residue;

R^3, which may be the same or different on each aryl group, is -H, halogen, hydrocarbyl, aryl, hydrocarbylaryl or a substituted derivative thereof;

X is -OH, -OM (wherein M is a salt forming metal), or a group containing an acrylate or methacrylate functional group;

Z is O or S or NOH;

or a polymer of a compound of the formula I in which X is a group containing an acrylate or methacrylate functional group.

Preferred embodiments of the invention include:

antifungal agents of formula I as defined above wherein m is at least 1 and X is -OH or -OM,

more particularly wherein n = p = o,

preferably wherein m is 4-8 and X is -OK,

most preferably wherein m is 8;

antifungal agents of formula I wherein m = n = o and R' is $CH_2CH_2NH_2$ HCl;

antifungal agents of formula I wherein R^2 is a pyrrole group;

antifungal agents of formula I wherein Z is NOH; and

antifungal agents of formula I wherein m = p = o, n = 4, and a = o.

The term "hydrocarbyl" as used herein means aliphatic hydrocarbyl including alkyl, alkenyl and alkynyl and also includes alkylene and alkenylene groups in the case where R^4 and R^5 together form a cycloaliphatic ring. Hydrocarbyl groups shall preferably contain from 1 to 12 carbon atoms, more preferably from 1 to 5 carbon atoms, and aryl and hydrocarbylaryl groups shall preferably have from 6 to 20 carbon atoms, more preferably from 6 to 10 carbon atoms. Hydrocarbyl groups are preferred, especially alkyl or alkenyl groups.

A substituted derivative of the foregoing may suitably be substituted with one or more halo groups or radicals containing nitrogen or substituted or interrupted by one or more oxo groups. Radicals containing nitrogen may or may not form part of a heterocyclic ring; a suitable radical may contain an amino or amide group, or may be a heterocyclic ring which may be saturated or unsaturated, aliphatic or aromatic, for example a 5- or 6-membered ring containing 1 or 2 nitrogen atoms. Reference is directed to U.S. Patent 4,882,449 Harris, the contents of which are incorporated herein by reference. Halogen may be chlorine, bromine, fluorine or iodine.

The preparation of calixarene derivatives is known and is described, for example, in C. Gutsche et. al., Acc. Chem. Res., 16, 161-170 (1983); in U.S. Patents 4,556,700 Harris et. al., 4,866,198 Harris, and 4,882,449 Harris and in J. Inclusion Phenomena 2 199-206 (1984) D. Reidel Publishing Company; the appropriate disclosures of all of which are incorporated herein by reference.

The preparation of aryl calixarene derivatives is described in European Patent Publication No. 0,259,016 (Application No. 87 306 963.7) and equivalent applications in other countries.

Mixed functionality calixarene derivatives are described in European Patent Publication No. 0,196,895 A2 and U.S. Patent 4,642,362 Harris et. al. When m is greater than or equal to 2 in the compounds of formula I, the aryl groups having the $-OCH_2C(O)X$ side chain may be interspersed around the ring between the aryl groups having the other side chains.

In the oxacalixarene derivatives of formula VI when a is 1 in more than one aryl group, the methylene and ether bridges may or may not alternate within the oxacalixarene molecule.

Oxacalixarene compounds may be readily synthesised by methods described in C. Gutsche et. al., J. Am.Chem. Soc. 103, 3782 (1981); B. Dhawan et. al., J. Org. Chem., 48, 1536 (1983), U.S. Patent 4,098,717 Buriks et. al., U.S. Patent 4,933,407 Harris et. al., and European Patent Publication No. 0,309,291 (Application No. 88 308 897.3) the appropriate disclosures of which are incorporated herein by reference.

Calixarene and oxacalixarene derivatives may usefully be polymer-bound by methods described in U.S. Patent 4,642,362 Harris et. al., or 4,699,966 Harris et. al., or by methods analogous to those described for crown ethers in U.S. Patent 4,447,585 Parker or Tetrahedron 36 461-510 (1980). The derivatives may also be silica gel bound by methods analogous to those described in J. Incl. Phenomena 7 127-136 (1989) or J. Chem. Soc. Chem. Comm. 812 (1988).

If X is a group containing an acrylate or methacrylate functional group, said group is preferably of the formula

$$-O(CH_2)_q\ O\underset{\displaystyle\overset{\|}{O}}{C}\ \underset{\displaystyle\overset{|}{R''}}{C}=CH_2$$

wherein q = an integer 2-10 and
R" is H or CH_3.

Due to the (meth)acrylate functionality, such compounds are capable of free radical polymerisation.

The invention also relates to a method for the synthesis of compounds of formula V defined above wherein pyrogallol is condensed with an aldehyde. Preferably the resulting tetramer is halogenated. This tetramer may be converted to its alkyl acetate derivative and optionally base - hydrolysed to give a potassium acetate derivative. The potassium salt may then be converted to the acid and subsequently to the ammonium salt.

In particular, pyrogallol is condensed with an aldehyde in equimolar quantities in refluxing 37% aqueous HCl/ethanol 1/4 by volume following the method of J. Holmes et al, European Patent No. 400,773 5th December 1990 for I.C.I. The precipitated tetramer is washed with a minimum quantity of cold ethanol then brominated with one equivalent of bromine in $CHCl_3$ then converted to its ethyl acetate derivative by treatment with 24 equivalents of ethyl bromoacetate and 18 equivalents of anhydrous potassium carbonate in refluxing dry acetone following the method of S. J. Harris et al , U.S. Patent No. 4,556,700 but with a shorter reaction time, for 48 hours following which the unpurified ester is hydrolysed with an equal weight of potassium hydroxide in refluxing ethanol for 2 hours to give the potassium acetate derivative which is filtered off under nitrogen and washed with a minimum quantity of cold ethanol.

Conversion to acid is accomplished by addition of a minimum quantity of 37% aqueous HCl and washing the precipitate with a minimum quantity of cold water.

Conversion to the ammonium salt is simply by addition of excess 25% aqueous analar NH_4OH at 50^oC in an oven overnight.

The invention also provides anti-bacterial, anti-fungal, anti-viral and anti-cancer agents comprising as active ingredient calixarene or oxacalixarene derivatives of formulae I, II, III, IV, V or VI.

The invention further provides pharmaceutical compositions comprising a pharmaceutically effective amount of any of the above defined compounds, either singly or in combination. The invention also provides use of any of the above defined compounds, either singly or in combination, in the preparation of a medicament for the treatment of bacterial infection, fungal infection or viral infection, particularly HIV-1, HIV-2 or SIV infection. The invention also provides a method of medical treatment comprising administering a therapeutically effective amount of any of the above defined compounds to a patient, either singly or in combination.

Particularly preferred compounds with antibiotic activity are those of Formula I in which R^2 is CH_2CO_2H, m is 0, n is 4, X is hydrogen and R^1 is hydrogen.

Also preferred are compounds of Formula I in which

(a) R^2 is CH_2CO_2K, m is 0, n is 5, X is hydrogen and R^1 is Br; or

(b) R^2 is $CH_2\overset{\overset{O}{\|}}{C}N\begin{matrix} \diagup R^4 \\ \diagdown R^5 \end{matrix}$

n is 4, m is 0, X is hydrogen, R^1 is t-butyl and R^4 is the same as R^5 and is $CH_2CH_2OCH_3$, $(CH_2)_7CH_3$, $(CH_2)_9CH_3$; or

(c) R^2 is $CH_2\overset{\overset{O}{\|}}{C}N\begin{matrix} \diagup R^4 \\ \diagdown R^5 \end{matrix}$

n is 3, m is 1, X is hydrogen, R^1 is t-butyl, and R^4 is the same as R^5 and is CH_2CH_3; or

(d) R^2 is $CH_2\overset{\overset{O}{\|}}{C}N\begin{matrix}\diagup R^4\\ \diagdown R^5\end{matrix}$

n is 3, m is 1, X is hydrogen, R^1 is Br and R^4 is the same as R^5 and is $(CH_2)_7CH_3$; or

(e) R^2 is $CH_2\overset{\overset{O}{\|}}{C}N\begin{matrix}\diagup R^4\\ \diagdown R^5\end{matrix}$

n is 2, m is 2, X is hydrogen, R^1 is t-butyl and R^4 is the same as R^5 and is $CH_2CH_2OCH_3$;
or

(f) R^2 is $CH_2\overset{\overset{O}{\|}}{C}N\begin{matrix}\diagup R^4\\ \diagdown R^5\end{matrix}$

n is 7, X is hydrogen, R^1 is ethyl and R_4 is the same as R^5 and is CH_2CH_2OH.

Particularly preferred compounds with anti-cancer activity are compounds of Formula I in which

(a) R^1 is CH_2CH_3, R^2 is $OCH_2\overset{\overset{O}{\|}}{C}\text{-}OCH_2CH_2O(CH_2)_3CH_3$, n is 7, m is 0 and X is hydrogen; or

(b) R^1 is t-butyl, n is 4, m is 0, X is hydrogen, R^2 is

$OCH_2\overset{\overset{O}{\|}}{C}N\begin{matrix}\diagup R^4\\ \diagdown R^5\end{matrix}$

where R^4 is the same as R^5 and is CH_2CH_3, $CH_2CH_2OCH_3$; or 

(c) R^1 is t-butyl, n is 5, m is 0, X is hydrogen, R^2 is

$OCH_2\overset{\overset{O}{\|}}{C}N\begin{matrix}\diagup R^4\\ \diagdown R^5\end{matrix}$

where R^4 is the same as R^5 and is CH_2CH_3; or

(d) R^1 is t-butyl, n is 2, m is 2, X is hydrogen, R^2 is

$OCH_2\overset{\overset{O}{\|}}{C}N\begin{matrix}\diagup R^4\\ \diagdown R^5\end{matrix}$ where R^4 is the same as R^5 and is CH_2CH_3.

(e) R^1 is t-butyl, n is 4, m is 0, R^4 is phenyl and R^5 is cholesterol.

The invention will now be described in greater detail with reference to the following Examples. The formulae for the compounds referred to in the Examples are given in the section entitled "Structural Formulae".

PARENT (OXA) CALIXARENES

Example 1: - Calix-(7)-arene

P-T-Butylcalix-7-arene was prepared following the procedure of Y. Nakamoto, S. Ishida Makromol, Chem Rapid Commun 3 p.705 1982, then dealkylated with aluminium chloride, phenol and toluene following the procedure by C. D. Gutsche and L. Lin in Tetrahedron 42 6 p. 1633 1986 to give calix-7-arene as a very pale orange solid in 80% yield.

Example 2: P-Nitrocalix-7-arene

Calix-7-arene from the above example was converted to its p.nitro derivative by treating with concentrated sulphuric acid then nitric acid following the method of S. Shinkai, T. Tsubaki, T. Sone and O. Manabe Tetrahedron Letters 26 (28) p. 3343 1985 and was obtained as a pale yellow solid in overall 70% yield.

Example 3: P-Bromocalix-5-arene

P-T-Butylcalix-5-arene was prepared following the method of A. Ninagawa and H. Matsuda Makromol Chem Rapid Commun 3 p.65 1982 and dealkylated with aluminium chloride, phenol and toluene as in the first example to give calix-5-arene as a pale pink solid. 1.0g (0.0019 mole) of calix-5-arene in 25 ml chloroform was treated overnight with 1.28g, (0.008)mole bromine in 5 ml chloroform at room temperature with stirring under nitrogen. Subsequent removal of all volatiles gave 1.7g, 97% yield of p-bromo-calix-5-arene as a pale pink solid.

Example 4: P-Bromocalix-6-arene

This compound was prepared as a pale orange solid following the method of Example 3. The p-t-butylcalix-6-arene starting material was prepared

following the method of C.D. Gutsche, B. Dhawan, K. H. No and R. Muthukrishnan, J Am Chem Soc. 103 p. 3782 1981.

Example 5: P-Octadecanoylcalix-7-arene

Calix-7-arene prepared as in Example 1 was treated with octadecanocyl chloride following the method of Y. Nakamoto, G. Kallinowski, V. Bohmer and W. Vogt. Langmuir 5 p.116 1989 in 80% yield as an off-white solid p-octadecanoylcalix-7-arene.

Example 6: P-Octadecanoylcalix-4-arene

P-T-Butylcalix-4-arene was prepared following the method of C.D. Gutsche, B. Dhawan, K. H. No and R. Muthukrishnan. J Am Chem Soc 103 p. 3782 1981 then dealkylated following the procedure given in Example 1. Treatment with octadecanoyl chloride as in Example 5 gave p-octadecanoylcalix-4-arene as a pale-grey solid in 75% yield I.r.ν 3256M OH, 1677 SC=O cm^{-1}.

Example 7: P-Octadecanoylcalix-8-arene

P-T-Butylcalix-8-arene was prepared following the method of C. D. Gutsche, B. Dhawan, K. H. No and R. Muthukrishnan. J Am Chem Soc 103 p. 3782 1981 then dealkylated following procedure given in Example 1. The calix-8-arene was then converted to p-octadecanoylcalix-8-arene in 70% yield as a grey solid following the procedure of Example 5. I.r.ν 3284 MOH, 1678 SC=Ocm^{-1}.

Example 8: P-Sodiumsulphonatecalix-4-arene

P-Sodiumsulphonatecalix-4-arene was prepared following the method of J. Atwood, A. W. Coleman, H. Zhang and S. G. Bott, Journal of Inclusion Phenomona and Molecular Recognition 7 p.203 1989.

Example 9: P-Bromodihomooxacalix-4-arene

P-T-Butyldihomooxacalix-4-arene was prepared by the method of B. Dhawan and C.D. Gutsche J. Org Chem 48 p. 1536 1983, then dealkylated by the method of Ex. 1 but reaction time was 10min instead of 1h and at $0^{o}C$ instead of room temp., to give dihomooxacalix-4-arene. Treatment in chloroform with bromine

at room temp. following the method of Ex. 3 gave p-bromodihomooxcalix-4-arene as a pale orange solid.

Example 10: P-Hydroxycalix-4-arene

P-Methoxycalix-4-arene from Parish Chemical was treated with iodotrimethylsilane following the method of M. E. Jung and M. A. Lyster J. Org. Chem. 42 (23) p. 3761 1977 to give p-hydroxycalix-4-arene as a grey solid in 50% yield.

Example 11: O,O'-Dibromo-p-hydroxycalix-4-arene

Treatment of p-hydroxycalix-4-arene prepared in above Example 10 with 8 equivalents bromine instead of 4 as in Example 3 in chloroform gave O,O'-dibromo-p-hydroxycalix-4-arene as a pale brown solid in 98% yield.

ESTER MODIFIED CALIXARENES

TETRAMERS

Example 12, Compound 12:

P-T-Butylcalix-4-arene tetraacetic acid was prepared following the method of S. J. Harris U.S. Patent 4,882,449 November 21 1989 by hydrolysis of its ethyl acetate derivative with potassium hydroxide but with ethanol only (no water) which after neutralisation with aqueous HCl and filtering off and drying was treated with excess thionyl chloride under reflux for 2 hours after which all volatiles were removed to give p-t-butylcalix-4-arene tetraacetyl chloride which was not purified on account of its moisture sensitivity. To 1.0g (0.0011 mole) of this acid chloride in 10 ml dry tetrahydrofuran (THF) was added 0.68g (0.0044) mole

$HOCH_2CH_2\overset{\overset{O}{\|}}{P}(OCH_3)_2$ obtained from Fluka and 0.35g (0.0044) mole pyridine in 5 ml dry THF. The reaction mixture became cloudy. The reaction mixture was stirred at room temperature overnight after which all volatiles were removed under reduced pressure. Treatment of the residue with 2% aqueous HCl and dichloromethane gave, after washing the organic layer with dried $MgSO_4$ and removal of volatiles 88% 1.38g yield of Compound 12 as a colourless solid.

Example 13, Compound 13:

P-Nitrocalix-4-arene tetracetic acid was prepared in the following way. Cone conformational calix-4-arene t-butyl acetate derivative was prepared following the method of S. J. Harris, M. A. McKervey, G. Svehla and D. Diamond U.S. Patent 5,132,345 July 21 1992.

Thus 0.5g of cone Compound 13a was dissolved in 10 ml glacial acetic acid which was cooled to $0^{o}C$. To this stirred solution was added a mixture of 5 ml concentrated nitric acid and 5 ml concentrated sulphuric acid dropwise to the solution which attained a purple colour. Stirring was continued for 2 hours at $0^{o}C$ during which the reaction mixture thickened. The reaction mixture was stirred overnight at room temperature to give a clear green solution which was subsequently cooled to $0^{o}C$ and 25 ml water was added dropwise to give an off-white solid precipitate which was filtered off and allowed to dry overnight after washing with minimum amount cold water to give 0.4g cone p-nitrocalix-4-arene tetracetic acid. I.r. ν 1450cm^{-1}M NO_2 stretching band cm^{-1} cone Compound 13b. Treatment of 0.4g of this tetracid with 5 ml thionyl chloride under reflux for 2 hours following the method of Example 12 gave 0.43g Compound 13c as a pale yellow solid after removal of all volatiles under reduced pressure. To this solid 0.43g (0.00047mole) in 5 ml dry THF was added 0.29g(0.0019 mole) $HOCH_2CH_2$ $P{=}O(OCH_3)_2$ and 0.15g dry pyridine in 2 ml dry THF. The reaction mixture went cloudy. Subsequent work up following the method of Example 12 gave the title compound in 80% yield as an orange-brown solid.

Example 14, Compound 14:

P-Hydroxycalix-4-arene was treated with ethyl bromoacetate, and anhydrous potassium carbonate in refluxing dry acetone for 5 days following the method of S. J. Harris, M. A. McKervey, D. P. Melody, J. Woods, and J. M. Rooney in U.S. Patent 4,556,700 December 3rd 1985 to give the title product as a heavy pale yellow oil in 80% yield.

Example 15, Compound 15:

Similarly treatment of 0,0-dibromo-p-hydroxycalix-4-arene prepared in Example 11 with ethyl bromoacetate potassium carbonate in refluxing acetone for 5 days following the method given in Example 14 gave the title product as

a pale brown oil in 85% yield. I.r. ν 1755S C=O cm^{-1}.

Example 16, Compound 16:

1g Compound 16a was treated with 1g potassium hydroxide in ethanol 10 ml which was refluxed for 2 hours, volatiles were then removed and the residue was treated with HCl following the method of S. J. Harris U.S. Patent 4,882,449 to give Compound 16b. Treatment with thionyl chloride following the method of Example 12 followed by treatment with $HOCH_2CH_2P{=}O(OCH_3)_2$ and pyridine in THF following the method of Example 12 gave Compound 16 as a pale brown-yellow solid in 79% yield.

Example 17, Compound 17:

During the preparation of Compound 17a in J. Am Chem Soc. 111 p.8681 1989 by S. J. Harris, F. Arnand-Neu, E. M. Collins, M. Deasy, G. Ferguson, B. Kaitner, A. J. Lough, M. A. McKervey, E. Marques, B. L. Ruhl, M. J. Schwing-Weill and E. M. Seward, a 10% quantity of side product was obtained whose X-ray crystallographic data (unpublished work) revealed it to be Compound 17b mp152-3^{o}C. This product was subsequently treated with ethyl bromoacetate, potassium carbonate in anhydrous acetone following the procedure described in Example 14 to give the title product as pale yellow solid.

Example 18, Compound 18:

P-Bromocalix-6-arene prepared in Example 4 was treated with ethyl bromoacetate, anhydrous potassium carbonate in refluxing dry acetone following the method described in Example 14. Subsequent conversion of the Compound 18a to acid following the method in Example 12 and thence acid chloride also in Example 12, then treatment with methoxyethanol in the presence of pyridine in dry THF following the method given in Example 12, gave the title product in 80% yield as a pale yellow solid.

Example 19, Compound 19:

P-t-Butylcalix-8-arene was dealkylated following the method in Example 7, then brominated following the method given in Example 3, then etherified with ethyl bromoacetate and potassium carbonate in refluxing acetone following the method given in Example 14, then converted to its methoxyethyl acetate

derivative following Example 18 to give the title product as a pale yellow solid I.r. ν 1751 S C=Ocm^{-1}.

Example 20, Compound 20:

Calix-8-arene was obtained from dealkylation of its t-butyl derivative following the method in Example 7, then etherified with ethyl bromoacetate and potassium carbonate in refluxing acetone following the method in Example 14, then converted to its methoxyethyl ester derivative following the method of Example 18.

Example 21, Compound 21:

Calix-7-arene prepared as in Example 1 was converted to its methoxyethyl ester derivative following the method of Example 18. Product was obtained as a colourless waxy solid I.r. ν 1755 S C=Ocm^{-1}.

Example 22, Compound 22:

Calix-7-arene prepared as in Example 1 was converted to its P-bromocalix-7-arene by treatment with bromine in chloroform following the method in Example 3 and etherified with ethyl bromoacetate as in Example 14 and converted to its methoxyethyl acetate derivative following the method given in Example 18, as a pale brown solid I.r. ν 1756S C=Ocm^{-1}.

Example 23, Compound 23:

P-T-Butylcalix-7-arene was prepared following the method in Example 1, then converted to its methoxy ethyl ester derivative following the method in Example 18 and obtained as a colourless solid.

Example 24, Compound 24:

P-Nitrocalix-7-arene prepared following the method of Example 2 was treated with ethyl bromoacetate in the presence of anhydrous potassium carbonate in refluxing acetone following the method of Example 14, then converted to its methoxyethyl acetate derivative following the method of Example 18. The product was obtained as a pale-brown solid I.r. ν 1731S C=O, 1461 S NO_2cm^{-1}.

Example 25: P-Octadecanoylcalix-7-arene Hepta-ethyl Acetate Derivative

P-Octadecanoylcalix-7-arene was prepared as in Example 5, then treated with ethyl bromoacetate in the presence of anhydrous potassium carbonate in refluxing acetone following the method of Example 14 to give the title product in 85% yield as a yellow solid.

Example 26: P-Octadecylcalix-7-arene Hepta-ethyl Acetate Derivative

P-Octadecanoylcalix-7-arene prepared as in Example 5 was treated with triethylsilane/trifluoroacetic acid following the method of Y. Nakamoto, G. Kallinowski, V. Bohmer and W. Vogt, Langmuir 5 p.116 1989 to give p-octadecylcalix-7-arene which was then converted to its ethyl acetate derivative by treatment with ethyl bromoacetate in the presence of anhydrous potassium carbonate in refluxing dry acetone following the method of Example 14 to give the title product as a yellow solid I.r. ν 1756S C=Ocm^{-1}.

Example 27: P-Octadecylcalix-7-arene Hepta-methoxy Ethyl Acetate Derivative

The ethylacetate p-octadecylcalix-7-arene was converted to its methoxyethyl acetate following the method of Example 18 to give the title product as a pale brown oil I.r. ν 1757S C=Ocm^{-1}.

Example 28: P-Octadecanoylcalix-7-arene Hepta-methoxy Ethyl Acetate Derivative

The ethyl acetate of p-octadecanoylcalix-7-arene from Example 25 was converted to its methoxyethyl derivative following the method of Example 18. I.r. ν 1757S (O)C=O, 1681S C=Ocm^{-1}.

Example 29: P-Ethylcalix-7-arene Hepta-ethyl Acetate Derivative

P-Ethylcalix-7-arene was prepared following the method of Z. Asfari and J. Vicens Makromol Chem Rapid Commun 10 p.177 1989. Treatment with ethyl bromoacetate in the presence of anhydrous potassium carbonate in refluxing dry acetone following the procedure of Example 14 gave the the title product in 82% yield as a heavy colourless oil. The procedure followed is given in more detail in U.S. Patent 5,132,345 July 21 1992 by S. J. Harris, M. A. McKervey, G. Svehla and D. Diamond. Chromatography on basic alumina employing

dichloromethane as eluent gave the title product as a colourless oil which solidified on standing, mp46-7^{o}C I.r. ν C=O (S) 1750. Elemental Analysis calculated for $C_{91}H_{112}O_{21}$C=70.89, H=7.32; Found C=70.99, H 7.48%.

Example 30: P-Ethylcalix-7-arene Hepta-methoxyethyl Acetate Derivative

P-Ethylcalix-7-arene hepta-ethyl acetate from Example 29 was converted to its methoxyethyl acetate derivative in 90% yield as a colourless oil by refluxing 24 hours with excess methoxyethanol in the presence of catalytic (few mg per 1g starting material) quantity of p-toluene sulphonic acid to give the title product after removal of all volatiles under reduced pressure.

Example 31: P-Ethylcalix-7-arene Hepta-hydroxyethyl Acetate Derivative

P-Ethylcalix-7-arene hepta-ethyl acetate from Example 29 was converted to its hydroxyethyl acetate derivative as a colourless oil by treatment with ethylene glycol following the method of Example 30, obtained in 89% yield I.r. ν 3440 MOH, 1751S C=Ocm^{-1}.

Example 32: P-Ethylcalix-7-arene Hepta-n-butyloxyethyl Acetate Derivative

P-Ethylcalix-7-arene hepta-ethyl acetate from Example 29 was converted to its n-butyloxyethyl acetate derivative as a pale yellow oil via its acid, and acid chloride and final treatment with n-butyloxyethanol in THF in the presence of pyridine following the method of Example 12.

Example 33: P-Ethylcalix-7-arene Hepta-ethoxyethyl Acetate Derivative

P-Ethylcalix-7-arene hepta-ethyl acetate from Example 29 was converted to its ethoxyethyl acetate as a colourless oil by transesterification with ethoxy-ethanol in the presence of p-toluene sulphonic acid following the method of Example 30 to give the title product as a colourless oil which solidified on standing.

Example 34: P-Ethylcalix-7-arene Hepta-methoxyethoxy-ethoxyethyl Acetate Derivative

P-Ethycalix-7-arene hepta-ethyl acetate from Example 29 was hydrolysed to its acid following the method of Example 12, followed by conversion to its

acid chloride in the same Example. Treatment then with methoxy ethyoxyethoxyethanol in THF in the presence of pyridine following the method of Example 12 gave title to the product as an orange oil solidifying on standing.

Example 35: P-Ethylcalix-7-arene Hepta-methylthioethyl Acetate Derivative

P-Ethylcalix-7-arene hepta-ethyl acetate from Example 29 was converted to its methylthioethyl acetate derivative as a pale yellow oil by treatment of its acid chloride derivative with methylthioethanol following the method of Example 12.

ACID/ACID SALT MODIFIED CALIXARENES

Example 36: P-Octadecylcalix-4-arene Potassium Acetate Derivative

P-Octadecylcalix-4-arene was prepared following the method of Y. Nakamoto, G. Kallinowski, V. Bohmer and W. Vogt, Langmuir 5 p.116 1989. I.r.ν 3172 M OHcm^{-1}. Treatment with ethyl bromoacetate and anhydrous potassium carbonate in refluxing dry acetone following the method of Example 14 gave p-octadecylcalix-4-arene tetra-ethyl acetate as a pale brown solid in 60% yield, mp 49-51^{o}C. I.r.ν cm^{-1} 1757S br C=Ocm^{-1} following chromatographic purification on neutral alumina with 50% petroleum ether 50% dichlomethane. Elemental analysis calculated for $C_{116}H_{192}O_{12}$-CH_2CL_2 C=75.40, H=10.41, Found 74.70, H=10.00%.

All 0.37g (0.0002 mole) of this compound was then refluxed for 2 hours with 0.40g 0.0071 mole potassium hydroxide in 5 ml ethanol. After cooling the pale brown potassium acetate calixarene was filtered off and obtained in 90% yield as a pale brown waxy solid.

Example 37: P-Bromocalix-4-arene Tetra-potassium Acetate Derivative

P-Bromocalix-4-arene was prepared following the method of Example 3. To 0.55g (0.00074 mole) of p-bromocalix-4-arene was added 0.61g (0.0044 mole) anhydrous potassium carbonate and 0.98g (0.0059 mole) ethyl bromoacetate and 10ml dry analar acetone and the entire was stirred under reflux for 168 hours. After removal of solvent the product was taken up into 10 ml dichloromethane which was washed with 3% aqueous sulphuric acid, then with

water, after which the organic layer was dried with dried magnesium sulphate. Removal of solvent gave 0.81g (ca 100% yield) product which was recrystallised from ethanol to give 0.71g 89% yield pure ethyl acetate product mp 137.5-142°C.

I.r.ν 1755 S, 1735 S C=Ocm^{-1}. Elemental Analysis required for $C_{44}H_{44}O_{12}Br_4$; C=48.75, H=4.06, Br=29.48; Found C=49.12, H=4.16, Br=29.30%.

All this compound was then hydrolysed with potassium hydroxide as in Example 36 to give a pale yellow solid the title product in 80% yield.

Example 38 P-Bromocalix-4-arene Tetra-acetic Acid Derivative

The tetra-potassium acetate salt of p-bromocalix-4-arene prepared in Example 37 was treated with 37% HCl and then washed with ice cold water and dried overnight at room temperature to give the title product as a pale yellow solid.

Example 39 P-Nitrocalix-4-arene Tetra-acetic Acid Derivative (Cone Conformational)

The title compound was prepared as described in Example 13 as a red brown solid.

Example 40: P-Nitroxcalix-4-arene Tetra-potassium Acetate Derivative Cone

Treatment of p-nitrocalix-4-arene tetra-acetic acid prepared in Example 39 was treated with 4 equivalents of potassium hydroxide in ethanol to give the title product in quantitative yield, after removal of volatiles under reduced pressure, as a pale yellow solid which was readily water soluble.

Example 41: P-Nitrocalix-4-arene Acetic Acid Derivative (Partial-Cone Conformational

Partial cone calix-4-arene tetra-t-butyl acetate derivative prepared following the procedure of S. J. Harris, M. A. McKervey, G. Svehla and D. Diamond U.S. Pat. 5,132,345 July 21 1992, was treated following the method of Ex. 13 with concentrated nitric acid/concentrated sulphuric acid to give

partial cone p-nitrocalix-4-arene acetic acid as a pale yellow solid. I.r. ν 1450 M Nitro stretching band cm^{-1}.

Example 42: P-Nitrocalix-4-arene Tetra-potassium Acetate Derivative Partial Cone

Partial cone p-nitrocalix-4-arene tetra-acetic acid from Ex. 41 was treated with potassium hydroxide in ethanol following the method in Ex. 40 to give the title compound as a yellow solid readily soluble in water.

Example 43: Cone-P-Nitrocalix-4-arene Tetra-ammonium Acetate Derivative

Cone conformational p-nitrocalix-4-arene tetra-acetic acid from Example 39 was treated with excess aqueous analar 25% ammonium hydroxide overnight in a 50^{o}C oven to give a pale brown readily water soluble solid as the title product.

Example 44: P-Sodium Sulphonate Calix-4-arene Tetra-methyl Ether Derivative

This compound was prepared following the method of Chemistry Letters p.1033 1985.

Example 45: P-Potassium Sulphonate-calix-4-arene Tetra-potassium Acetate Derivative

P-Sodiumsulphonatecalix-4-arene was prepared following the method in Example 8. Treatment with ethyl bromoacetate and anhydrous potassium carbonate in refluxing analar dry acetone following the procedure of Example 14 gave p-sulphonic acid calix-4-arene tetra-ethyl acetate derivative upon acid work up. Treatment with potassium hydroxide in ethanol following the method in Example 36 gave the title compound as a colourless solid upon removal of all volatiles under reduced pressure.

Example 46: P-Sulphonic Acid Calix-4-arene Tetra-acetic Acid Derivative

Treatment of p-potassium sulphonate calix-4-arene tetra-potassium acetate with minimum quantity 37% aqueous HCl and washing resulting solid with a minimum quantity of cold water gave a solid colourless title product in quantitative yield after air drying overnight.

Example 47: P-Potassium Oxyacetatecalix-4-arene Tetra-potassium Acetate Derivative

P-Ethyloxyacetatecalix-4-arene tetra-ethyl acetate prepared as in Example 14 was treated with excess potassium hydroxide in ethanol following the procedure of Example 36, giving the title product in 90% yield as a pale orange solid after removal of all volatiles, which was readily water-soluble.

Example 48: P-Oxyacetic Acid Calix-4-arene Tetra-acetic Acid Derivative

P-Potassium oxyacetate calix-4-arene tetra-potassium acetate derivative prepared in Example 47 was treated with 37% aqueous HCl, then the resulting solid was washed with a minimum amount of cold water as in Example 46 to give colourless solid the title product in near quantitative yield.

Example 49: P-Ammonium Oxyacetate P-Calix-4-arene Tetra-ammonium Acetate Derivative

P-Oxyacetic Acid calix-4-arene tetra-acetic acid prepared in Example 48 was treated with excess 25% analar aqueous ammonium hydroxide following the method of Example 43 giving a colourless solid the title product in quantitative yield, which was readily soluble in water.

Example 50: O,O'-Dibromo-P-Potassium Oxyacetate Calix-4-arene Tetra-potassium Acetate Derivative

O,O'-Dibromo-p-ethyl acetate oxycalix-4-arene tetra-ethyl acetate derivative prepared in Example 15 was treated with potassium hydroxide in ethanol following the method of Example 36 to give the title product as a pale orange-brown solid in near quantitative yield, which was readily water soluble.

Example 51: O,O'-Dibromo-P-Oxyacetic Acid Calix-4-arene Tetra-acetic Acid Derivative

O,O'-Dibromo-p-potassium oxyacetate calix-4-arene tetra-potassium acetate derivative prepared in Example 50 was treated with 37% aqueous HCl to give the title product as a grey-yellow solid after washing with a minimum quantity of ice cold water following the method in Example 38.

ACID/SALT OXACALIXARENE DERIVATIVES

Example 52: P-T-Butyldihomooxacalix-4-arene Potassium Acetate Derivative

P-t-butyldihomooxacalix-4-arene tetra-ethyl acetate derivative was prepared following the method of S. J. Harris and M. G. MacManus, U.S. Patent 4,855,461 August 8 1989. Treatment with ethanolic potassium hydroxide following the method in Example 36 gave colourless solid the title product which was readily water soluble.

Example 53: P-T-Butyldihomooxacalix-4-arene Tetra-sodium Acetate Derivative

P-t-Butyldihomooxacalix-4-arene tetra-potassium acetate derivative prepared in Example 52 was neutralised with 37% aqueous HCl following the method of Example 38 to give the parent acetic acid derivative which was treated then with 4 equivalents NaOH in ethanol to give the title product after removal of all volatiles, as a colourless solid.

Example 54: P-T-Butyldihomooxacalix-4-arene Tetra-lithium Acetate Derivative

P-t-Butyldihomooxacalix-4-arene tetra-acetic acid derivative prepared in Example 53 was treated with 4 equivalents of lithium hydroxide following the method of Example 53 to give the title product as a colourless solid in quantitative yield.

Example 55: P-T-Butyldihomooxacalix-4-arene Tetra-rubidium Acetate Derivative

Replacement of lithium hydroxide with rubidium hydroxide in Example 54 gave the title product as a colourless solid.

Example 56: P-T-Butyldihomooxacalix-4-arene Tetra-caesium Acetate Derivative

Replacement of lithium hydroxide with caesium hydroxide in Example 54 gave the title product as a colourless solid.

Example 57: P-T-Butyldihomooxacalix-4-arene Di-magnesium Acetate Derivative

Replacement lithium hydroxide with magnesium hydroxide in Example 54 and with only 2 equivalents, gave the title product as a colourless solid.

Example 58: P-T-Butyldihomooxacalix-4-arene 1.33-aluminium Acetate Derivative

Replacement of lithium hydroxide with aluminium hydroxide in Example 54 but with only 1.33 equivalents gave the title product a colourless solid. I.r. ν 1635 S C=Ocm^{-1}.

Example 59: P-T-Butyldihomooxacalix-4-arene Di-calcium Acetate Derivative

Replacement of lithium hydroxide with calcium hydroxide in Example 54 and with only 2 equivalents gave the title product as a colourless solid. I.r. ν 1602 S C=Ocm^{-1}.

Example 60: P-T-Butyldihomooxacalix-4-arene Di-strontium Acetate Derivative

Replacement of lithium hydroxide with two equivalents of strontium carbonate in Example 54 gave the title product as a colourless solid I.r. ν 1606S C=Ocm^{-1}.

Example 61: P-T-Butyldihomooxacalix-4-arene Di-cupric Acetate Derivative

Replacement of lithium hydroxide by two equivalents cupric hydroxide in Example 54 gave the title product as a green solid I.r. ν 1611S C=Ocm^{-1}.

Example 62: P-T-Butyldihamooxacalix-4-arene Di-cobalt II Acetate Derivative

Replacement of lithium hydroxide by two equivalents Cobalt II hydroxide gave the title product as mauve solid.

Example 63: P-T-Butyldihomooxacalix-4-arene 1.33 indium III Acetate

Replacement of lithium hydroxide by 1.33 equivalents Indium III hydroxide gave the title product as a pale yellow solid.

Example 64: P-T-Butyldihomooxacalix-4-arene Di-zinc Acetate

Replacement of lithium hydroxide by two equivalents zinc carbonate gave the title product as an off-white solid. I.r. ν 1618S C=Ocm^{-1}.

Example 65: P-T-Butyldihomooxacalix-4-arene Tetra-ammonium Acetate

Treatment of P-t-Butyldihomooxacalix-4-arene tetra-acetic acid prepared in Example 53 was treated with aqueous NH_4OH following the method of Example 43 to give the title product as a colourless solid.

Example 66: P-T-Butyldihomooxacalix-4-arene Tetra-tetra-n-butyl-ammonium Acetate Salt

Treatment of P-t-Butyldihomooxicalix-4-arene tetra-acetic acid prepared in Example 53 with 4 equivalents of tetra n-butyl ammonium hydroxide in methanol, then heating in an oven at 50^{o}C overnight gave the title product as colourless sticky solid. I.r. ν 1614S C=Ocm^{-1}.

Example 67: Dihomooxacalix-4-arene Tetra-potassium Acetate Derivative

Dihomooxacalix-4-arene prepared by dealkylation of its t-butyl derivative of Example 9 was etherified with ethyl bromoacetate following the method of Example 14 to give its ethyl acetate derivative as a colourless oil. Treatment with potassium hydroxide in ethanol following the procedure of Example 36 gave the title product as a colourless solid.

Example 68: P-Nitrodihomooxacalix-4-arene Tetra-potassium Acetate

Dihomooxacalix-4-arene prepared in Example 67 was treated with concentrated sulphuric acid followed by nitric acid following the method in Example 2 to give p-nitrodihomooxacalix-4-arene as a yellow solid which was then treated with ethyl bromoacetate following the method in Example 14 to give its ethyl acetate derivative as a brown solid. This compound was then hydrolysed with potassium hydroxide in ethanol following the method of Example 36 to give the title product as a yellow solid with high water solubility.

Example 69: P-Bromodihomooxacalix-4-arene Tetra-potassium Acetate

P-Bromodihomooxacalix-4-arene was prepared following the method of Example 9 as a pale orange solid, then treated with ethyl bromoacetate following the method in Example 14 to give the ethyl acetate derivative as a pale yellow solid. Treatment with potassium hydroxide in ethanol following the method of Example 36 gave the title product as a yellow-brown solid.

Example 70: P-Bromodihomooxacalix-4-arene Tetra-acetic Acid

P-Bromodihomooxacalix-4-arene Potassium Acetate prepared in Example 69 was treated with 37% aqueous HCl to give the title product as a pale yellow solid following the method in Example 38.

Example 71: P-Bromodihomooxacalix-4-arene Tetra-ammonium Acetate

P-Bromodihomooxacalix-4-arene acetic acid prepared in Example 70 was treated with NH_4OH following the method in Example 43 to give the title product as a pale brown solid.

Example 72: P-Bromodihomooxacalix-4-arene Tetra-tetra-n-butyl-ammonium Acetate Salt

P-Bromodihomooxacalix-4-arene acetic acid prepared in Example 70 was treated with 4 equivalents of tetra-n-butyl ammonium hydroxide in methanol following the method in Example 66 to give the title product as pale brown solid.

Example 73: P-T-Butylcalix-5-arene Penta-potassium Acetate

P-t-Butylcalix-5-arene prepared following the method described in Example 3 was etherified with ethyl bromoacetate following the method in Example 14 to give its ethyl acetate derivative as a colourless solid. Treatment with ethanolic potassium hydroxide following the method in Example 36 gave the title product as a colourless solid I.r. ν 1601S C=Ocm^{-1}.

Example 74: P-Bromocalix-5-arene Penta-potassium Acetate

P-Bromocalix-5-arene prepared in Example 3 was etherified with ethyl bromoacetate following the method in Example 14. The ethyl acetate derivative obtained as a pale yellow solid. I.r. ν 1753 SC=Ocm^{-1} was treated with potassium hydroxide in ethanol following the method in Example 36 to give the title product as a pale yellow solid.

Example 75: P-Bromoxalix-5-arene Penta-acetic Acid

P-Bromocalix-5-arene penta-potassium acetate from Example 74 was treated

with HCl following the method of Example 38 to give the title product as a pale yellow solid.

Example 76: P-Nitrocalix-5-arene Penta-potassium Acetate

P-Nitrocalix-5-arene was prepared following the method of Example 2 from calix-5-arene prepared following the method in Example 3. Treatment with ethyl bromoacetate following the method in Example 14 gave p-nitrocalix-5-arene penta-ethyl acetate as a yellow solid which was in turn hydrolysed with potassium hydroxide in ethanol following the procedure in Example 36 to the title compound as an orange solid.

Example 77: P-Nitrocalix-5-arene Penta-acetic Acid

P-Nitrocalix-5-arene penta-potassium acetate from Example 76 was treated with aqueous HCl following the method given in Example 38 to give the title acid as a pale yellow solid as the title product.

Example 78: P-Bromocalix-6-arene Hexa-potassium Acetate

P-Bromocalix-6-arene hexa-ethyl acetate was prepared following Example 18. Subsequent hydrolysis with ethanolic potassium hydroxide following the method in Example 36 gave the title product as a pale yellow solid.

Example 79: P-Bromocalix-6-arene Hexa-acetic Acid

P-Bromocalix-6-arene hexa-potassium acetate from Example 78 was treated with aqueous HCl following the method in Example 38 to give a pale yellow solid as the title product.

Example 80: P-Bromocalix-6-arene Hexa-ammonium Acetate

P-Bromocalix-6-arene hexa-acetic acid prepared in Example 79 was treated with ammonium hydroxide following the method in Example 43 to give the title product as pale yellow solid.

Example 81: P-Bromocalix-6-arene Hexa-tetra-n-butyl Ammonium Acetate

P-Bromocalix-6-arene hexa-acetic acid prepared in Example 79 was treated

with 6 equivalents tetra-n-butyl ammonium hydroxide following the method in Example 66 to give the title product as pale brown solid.

Example 82: P-Nitrocalix-6-arene Hexa-potassium Acetate

P-Nitrocalix-6-arene was prepared following the method of S. Shinkai, T. Tsubaki, T. Sone and O. Manabe Tetrahedon Letters 26 p. 3343 1985 then etherified with hexa-ethyl bromoacetate following the method of Example 14 to give p-nitrocalix-6-arene ethyl acetate as a yellow solid. This product was converted to its potassium acetate salt as a pale orange-yellow solid by hydrolysis with ethanolic potassium hydroxide following the method in Example 36. I.r. ν 1608S C=Ocm^{-1}.

Example 83: P-Nitrocalix-6-arene Hexa-acetic Acid

P-Nitrocalix-6-arene hexa-potassium acetate from Example 82 was treated with HCl following the method of Example 38 to give the title product as a pale yellow solid.

Example 84: P-Nitrocalix-7-arene Hepta-potassium Acetate

P-Nitrocalix-7-arene hepta-ethyl acetate was prepared following the method in Example 24 which was then hydrolysed with ethanolic potassium hydroxide following the method of Example 36 to give the title product as a brown solid.

Example 85: P-Nitrocalix-7-arene Hepta-acetic Acid

P-Nitrocalix-7-arene hepta-potassium acetate from Example 84 was treated with HCl following the method in Example 38 to give the title product as a pale orange solid.

Example 86: P-Nitrocalix-7-arene Hepta-ammonium Acetate

P-Nitrocalix-7-arene hepta-acetic acid from Example 85 was treated with NH_4OH following the method of Example 43 to give the title product as a pale brown solid.

Example 87: P-Bromocalix-7-arene Hepta-potassium Acetate

P-Bromocalix-7-arene hepta-ethyl acetate was prepared following the method in Example 22 which was then treated with ethanolic potassium hydroxide following the method in Example 36 to give the title product as a pale yellow solid.

Example 88: P-Bromocalix-7-arene Hepta-acetic Acid

P-Bromocalix-7-arene hepta-potassium acetate prepared in Example 87 was treated with HCl following Example 38 to give the title product as pale yellow solid.

Example 89: P-Bromocalix-7-arene hepta-ammonium Acetate

P-Bromocalix-7-arene hepta-acetic acid prepared in Example 88 was treated with NH_4OH following the method in Example 43 to give the title product as pale brown solid.

Example 90: P-Bromocalix-7-arene Hepta-tetra-n-butyl-ammonium Acetate

P-Bromocalix-7-arene hepta-acetic acid prepared in Example 88 was treated with 7 equivalents of tetra-n-butyl-ammonium hydroxide in methanol following the method in Example 66 to give the title product as a brown solid.

Example 91: P-Octadecylcalix-8-arene Octa-potassium Acetate

P-Octadecanoylcalix-8-arene prepared in Example 7 was converted to p-octadecylcalix-8-arene by treatment with triethylsilane and trifluoroacetic acid in method quoted in Example 26 which was obtained as a grey waxy solid. This product was converted to its ethyl acetate derivative following the method in Example 14. I.r. ν 1750S C=Ocm^{-1} which was the converted to its the title product as a grey-brown solid by treatment with potassium hydroxide in ethanol following the method in Example 36.

Example 92: Calix-8-arene Octa-potassium Acetate

Calix-8-arene octa-ethyl acetate was prepared following the method in Example 20 and was subsequently treated with ethanolic potassium hydroxide

following the method in Example 36 to give the title product as a pale colourless solid.

Example 93: P-Bromocalix-8-arene Octa-potassium Acetate

P-Bromocalix-8-arene octa-ethyl acetate was prepared following the method in Example 19 which was then converted to its the title product as a pale yellow solid by treatment with ethanolic potassium hydroxide following the method of Example 36.

Example 94: P-Bromocalix-8-arene Octa-acetic Acid

P-Bromocalix-8-arene octa-potassium acetate prepared in Example 93 was treated with HCl following Example 38 to give the title product as pale yellow solid.

Example 95: P-Nitrocalix-8-arene Octa-potassium Acetate

Calix-8-arene was prepared following the method in Example 7, then treated with sulphuric acid, then nitric acid following the procedure in Example 2. The pale yellow p-nitrocalix-7-arene formed was treated with ethyl bromoacetate following the procedure in Example 14 to give its ethylacetate derivative as a brown solid. Treatment of this product with ethanolic potassium hydroxide following the method in Example 36 gave the title compound as a pale brown solid. I.r. ν 1610S C=Ocm^{-1}.

OPEN CHAIN PHENOL FORMALDEHYDE OLIGMERS (TETRAMERS)

Example 96, Compound 96:

The title compound was prepared following the procedure of C. D. Gutsche, B. Dhawan, K. H. No and R. Muthukrishnan, J Am Chem Soc 103 p.3782 1981, prior to its cyclisation to calix-4-arene in hot diphenylether.

Example 97, Compound 97:

The title compound was prepared as a brown solid by treating the t-butyl derivative prepared in Example 96 with $AlCl_3$, phenol and toluene following the procedure in Example 9.

Example 98, Compound 98:

The title compound was prepared as a pale yellow solid by treatment of its dealkylated derivative prepared in Example 97 with bromine in chloroform following the procedure in Example 9.

Example 99, Compound 99:

The the title product was prepared as a pale yellow solid by treatment of its p-bromo phenolic derivative prepared in Example 98, with ethyl bromoacetate following the procedure in Example 14.

Example 100, Compound 100:

The title compound was prepared as a pale brown solid by treatment of its ethyl acetate derivative prepared in Example 99 with ethanolic potassium hydroxide following the procedure in Example 36.

Example 101, Compound 101:

The title compound was prepared as a pale brown solid by treatment of its potassium salt derivative from Example 100 with HCl following the method in Example 38.

AMIDE FUNCTIONAL CALIXARENES

Example 102, Compound 102:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride prepared by the method in Example 12 was treated with Compound 102a and pyridine in THF at room temperature overnight to give the title product as an orange-red solid.

Example 103, Compound 103:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride as in Example 102 with aminotetrazole gave the title product as a colourless solid.

Example 104, Compound 104:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride as in Example 102 with p-dimethylaminoaniline gave the title product as a colourless solid.

Example 105, Compound 105:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride from Example 102 with $NH_2CH_2CH_2OP(O)(OCH_3)_2$ gave the title product as a colourless solid.

Example 106, Compound 106:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride from Example 102 with methylaminomethyl anthracene from Aldrich gave the title product as a yellow solid purified by chromatography on an alumina column (neutral) using dichloromehane as eluent obtained in 60% yield mp 158-161^{o}C. I.r.ν1658 C=0cm^{-1}.

Elemental analysis calculated for $C_{116}H_{116}N_4O_8$C=78.98, H=6.69, N=3.15; Found C=79.31, H=6.86, N=3.00% 'HNmr. $CDCl_3$ 8 ppm 1.12 S 36H $C(CH_3)_3$, 2.60 S, 12H NH_3C, 3.20 d 4HH_BArCH_2Ar, 5.05 S 8H OCH_2CON, 5.18 d 4H H_A ArCH_2Ar, 5.52 s 8H CH_2-anthracene, 6.83 s 8 HArH, 7.20-8.40 om 36H anthracene.

Example 107, Compound 107:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride from Example 102 with methyl ester of L,L-alanylalanine gave the title product as a pale yellow solid.

Example 108, Compound 108:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride from Example 102 with Compound 108a gave the title product as a colourless waxy solid.

Example 109, Compound 109:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride from Example

102 with Compound 109a gave the title product as an off-white solid.

Example 110, Compound 110:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride from Example 102 with

$$\begin{array}{l}\mathrm{D,L\text{-}NH_2CH\text{-}CO_2CH_3} \\ \qquad\qquad\quad\;\; | \\ \qquad\qquad\quad\; \mathrm{CH_2} \\ \qquad\qquad\quad\;\; | \\ \qquad\qquad\quad\; \mathrm{CHCl_2}\end{array}$$

furnished the title product as an off-white solid.

Example 111, Compound 111:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride from Example 102 with Compound 111a gave the title product as a colourless solid.

Example 112, Compound 112:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride from Example 102 with 2-D-alanyl-5-leucine-enkephalin-amide from Fluka gave the title product as a colourless solid.

Example 113, Compound 113:

Treatment of p-t-butylcalix-4-arene tetra-acetyl chloride from Example 102 with the methyl ester of glycylglycylhistidine gave the title product as a pale yellow solid.

Example 114, Cone Compound 114:

Cone P-Nitrocalix-4-arene tetra-acetyl chloride prepared following the method in Example 13 was reacted with bis-methoxyethylamine and pyridine in THF at room temperature overnight to give the title product as a pale brown solid. I.r. ν 1642S C=Ocm^{-1}.

Example 115, Cone Compound 115:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride prepared as in Example 114 was treated with the methyl ester of L,L-alanylalanine to give the title product as a pale yellow solid.

Example 116, Cone Compound 116:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride prepared in Example 114 was treated with

$$D,L\text{-}NH_2\text{-}\underset{\substack{|\\ CH_2\\ |\\ CHCl_2}}{CH}CO_2CH_3$$

to give the title product as pale yellow-brown solid.

Example 117, Cone Compound 117:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride prepared in Example 114 was treated with 2-D-alanyl-5-leucine enkephalin amide to give the title product as a yellow solid.

Example 118, Cone Compound 118:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride with Compound 118a furnished the title product as a red-brown solid.

Preparation 119, Cone Compound 119:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with Compound 119a prepared from its disodium salt gave the title product as a pale orange-brown solid.

Preparation 120, Cone Compound 120:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with

$$NH_2\text{-}CH\text{-}\underset{\substack{|\\ CO_2CH_3}}{CH}\text{=}CCL_2$$

gave the title product as pale brown solid.

Preparation 121, Cone Compound 121:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with Compound 121a gave the title product as a red-brown solid.

Example 122, Cone Compound 122:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with Compound 122a gave the title product as a yellow solid.

Example 123, Cone Compound 123:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with Compound 123a gave the title product as a yellow-brown solid.

Example 124, Cone Compound 124:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with the methyl ester of glycylglycylhistidine gave the title product as a pale yellow solid.

Example 125, Cone Compound 125:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with NH_2 CH_2 CH_2 $OP(O)(OCH_3)2$ gave the title product as a pale yellow solid.

Example 126, Cone Compound 126:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with Compound 126a gave the title product as pale brown solid.

Example 127, Cone Compound 127:

Treatment of cone p-nitrocalix-4-arene acetyl chloride of Example 114 with Compound 127a gave the title product as a pale brown solid.

Example 128, Cone Compound 128:

Treatment of cone p-nitrocalix-4-arene tetra-acetylchloride of Example 114 with methylaminomethyl anthracene gave the title product as a pale brown solid.

Example 129, Cone Compound 129:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with aminotriazole gave the title product as an orange-brown solid.

Example 130, Cone Compound 130:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with Compound 130a gave the title product as a red brown solid.

Example 131, Cone Compound 131:

Treatment of cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 with aminotetrazole gave the title product as pale yellow solid.

Example 132, Compound 132:

Compound 132a was prepared as Example 48 which was then converted to its acid chloride by treatment with thionyl chloride following the method in Example 12. This product was then reacted with bis-methoxyethylamine in Tetrahydrofuran with pyridine as in Example 114 to give the product as an off white solid.

Example 133, Compound 133:

Compound 133a prepared as in Example 132 was treated with aminotetrazole following the method in Example 131 to give the title product as a pale grey-brown solid.

Example 134, Compound 134:

Compound 133a prepared in Example 132 was treated with Compound 134a to give the title product as a brown solid.

Example 135, Compound 135:

P-Nitrocalix-6-arene hexa-acetic acid from Example 83 was converted to its acid chloride with thionyl chloride following the method of Example 12 and then converted to the title product as a bright red-brown solid by treatment

with Compound 134a as in Example 102.

Example 136, Compound 136:

P-Ethylcalix-7-arene hepta-acetyl chloride prepared in Example 32 was converted to the title product by treatment with $NH_2\ CH_2\ CH_2\ OH$ as a colourless oil. i.r. ν 3470 MOH, 1665 S C=O cm^{-1}.

Example 137, Compound 137:

P-calix-8-arene octa-potassium acetate prepared in Example 92 was treated with HCl following the method of Example 38 to give its acid which was then converted to its acid chloride with thionyl chloride following Example 12. This product was then treated with Compound 137a to give the title product as an off white solid.

Example 138, Compound 138:

The tetra-ethyl acetate derivative of p-t-butyldioxacalix-4-arene was prepared following method of US Patent 4855461 August 8 1989 by S.J. Harris and M.G. MacManus which was then hydrolysed to its acid via its potassium salt by treatment with alcoholic potassium hydroxide following the method of Examples 36/38. The acid derivative was converted to its acid chloride with thionyl chloride following the method in Example 12 which was then reacted with bis hydroxyethyl amine with pyridine in THF following the method in Example 102 to give the title product as an off-white solid.

ANTIBIOTIC DERIVATIVES

Example 139, Compound 139:

P-T-Butylcalix-4-arene tetra-acetyl chloride of Example 12 was treated with the cyclic polyene antibiotic from Sigma, Amphotericin B following the method in Example 102 to give the title amide product as a yellow solid.

Example 140, Compound 140:

P-t-butycalix-4-arene tetra-acetyl chloride of Example 12 was treated with the lactam antibiotic aminocephalosporanic acid from Fluka following the

method in Example 102 to give the title product as an off-white solid.

Example 141, Cone Compound 141:

Cone P-Nitrocalix-4-arene tetra-acetyl chloride of Example 114 was treated with Amphotericin B to give the title amide product as a red-brown solid.

Example 142, Cone Compound 142:

Cone P-Nitrocalix-4-arene tetra-acetyl chloride of Example 114 was treated with the cyclic polyene antibiotic pimaricin from Sigma to give the title product as a yellow solid.

Example 143, Cone Compound 143:

Cone P-Nitrocalix-4-arene tetra-acetyl chloride of Example 114 was treated with the cyclic polyene antibiotic Nystatin A_1 from Sigma to give the title product as a yellow solid.

Example 144, Cone Compound 144:

Cone p-nitrocalix-4-arene tetra-acetyl chloride of Example 114 was treated with the lactam antibiotic aminocephalosporanic acid from Fluka to give the title product as a pale orange-brown solid.

Example 145, Cone Compound 145:

Cone P-Nitrocalix-4-arene tetra-acetyl chloride of Example 114 was treated with the lactam antibiotic Ampicillin obtained from Wako Pure Chemical Industries Limited to give title product as a yellow solid.

Example 146, Cone Compound 146:

Cone P-Nitrocalix-4-arene tetra-acetyl chloride of Example 114 was treated with the lactam antibiotic aminopecillamic acid methyl ester (acid from Fluka) to give the title product as an orange-brown solid.

Example 147, Cone Compound 147:

Cone P-Nitrocalix-4-arene tetra-acetyl chloride of Example 114 was treated with the aminoglycoside antibiotic Sinefungin from Sigma to give the title product as a yellow solid.

Example 148, Compound 148:

P-Bromcalix-6-arene hexa-acetic acid was prepared by the method in Example 79 which was converted to its acid chloride with thionyl chloride as in Example 12. Subsequent reaction with the lactam antibiotic aminocephalosporanic acid gave the title product as a very pale-yellow solid.

Example 149, Compound 149:

P-Nitrocalix-6-arene hexa-acetic acid was prepared following the method in Example 83 which was converted to its acid chloride employing thionyl chloride as in Example 12. Subsequent treatment with the lactam aminocephalosporanic acid gave the title product as a yellow solid.

CYCLOTRIVERATHRYLENE DERIVATIVES

Example 150, Compound 150:

Cyclotriveratryline was converted to its parent phenolic derivative by treatment with boron tribromide in dry dichloromethane under nitrogen following the method of J.A. Hyatt, J Org Chem <u>43</u> (9) p1808 1978. This compound was subsequently reacted with 6 equivalents of bromine in dry chloroform under nitrogen at room temperature overnight to give after removal of volatiles its hexabromo derivative as a pale brown solid. This product was subsequently treated with ethyl bromoacetate following the method in Example 14 to give its ethyl acetate as a heavy brown oil which was then treated with ethanolic KOH following the method of Example 36 to give the title product as a dark-grey solid.

Example 151, Compound 151:

The Compound 150 prepared in Example 150 was treated with HCl to give its acid title product, following the method of Example 38, as a dark-brown solid.

Example 152, Compound 152:

The Compound 151 prepared in Example 151 was treated with NH_4OH following the method in Example 43 to give the title product as pale brown solid.

HOGBERG DERIVATIVES

Example 153, Compound 153:

Compound 153 was prepared following the method of A.G.S. Hogberg, J Org Chem 45 p4498 1980 as a very pale brown solid.

Example 154, Compound 154:

Compound 153 prepared in Example 153 was brominated with 4 equivalents of bromine in chloroform following the method in Example 3 to give to the title product as a red-brown solid.

Example 155, Compound 155:

Compound 153 from Example 153 was reacted with ethyl bromoacetate following the procedure in Example 14 to give the title product as a grey solid.

Example 156, Compound 156:

from Example 155 was treated with ethanolic KOH following the procedure of Example 36 to give the title product as a colourless solid.

Example 157, Compound 157:

Compound 156 from Example 156 was treated with HCl following the procedure in Example 38 to give title product as an orange solid.

Example 158, Compound 158:

Compound 154 from Example 154 was etherified with ethyl bromoacetate following the procedure of Example 14 to give the title product as a heavy

pale brown oil I.r. ν 1754S C=O cm^{-1}.

Example 159, Compound 159:

Compound 158 from Example 158 was treated with ethanolic potassium hydroxide following the procedure in Example 36 to give the title product as a very pale orange solid.

Example 160, Compound 160:

Compound 159 was treated with HCl following the method of Example 38 to give the title product as a grey solid.

Example 161, Compound 161:

Compound 155 prepared in Example 155 was treated with concentrated sulphuric acid/nitric acid following the method in Example 13 to give the title product as a red solid I.r. ν 1730 S C=O cm^{-1}.

Example 162, Compound 162:

Compound 161 from Example 161 was treated with ethanolic potassium hydroxide following the method of Example 40 to give the title product as a pale yellow-brown solid. I.r. ν 1610 S C=O cm^{-1}.

PYROGALLOL-ALDEHYDE CYCLIC TETRAMERS AND DERIVATIVES

Example 163, Compound 163:

The title compound was prepared as a pale pink solid by the reaction of n-butyraldehyde and pyrogallol in 1:4, 37% aqueous HCl to ethanol under nitrogen under reflux for 90 minutes following the method of J. Holmes and P. Tasker, European Patent Application EP 400,773 5th December 1990 assigned to ICI.

Example 164, Compound 164:

The cyclic tetramer of pyrogallol and butyraldehyde prepared in Example 163 was treated with 4 equivalents of bromine in chloroform following the

method of Example 3 to give a pale grey-brown solid as the title compound after removal of all volatiles.

Example 165, Compound 165:

Compound 163 from Example 163 was etherfied with 24 equivalents ethyl bromoacetate and 18 equivalents $K_2 CO_3$ in refluxing dry acetone for 48 hours following the method of Example 14 to give the title product as a pale yellow oil.

Example 166, Compound 166:

Compound 164 from Example 164 was etherified as in Example 165 to give the title product quantitatively as a pale orange heavy oil. I.r. ν 1753S, 1740 sh C=Ocm^{-1}.

Example 167, Compound 167:

Compound 165 prepared in Example 165 was treated with ethanolic potassium hydroxide following the method of Example 36 to give off white title product, which was soluble in water, as were all acid salts which follow.

Example 168, Compound 168:

Compound 167 prepared in Example 167 was treated with HCl then washed with water following the method of Example 38 to give the title product as an off-white solid which was not very water soluble nor were all subsequent acid derivatives.

Example 169, Compound 169:

Compound 166 prepared in Example 166 was refluxed with 1g (0.018 mole) potassium hydroxide in 10 ml refluxing absolute ethanol for 2 hours. After this time a pale brown suspension of solid had formed in the reaction mixture which was filtered off under nitrogen (the solid appears to rapidly pick up moisture from the air and turns to a blackish oil).

The pale brown solid was filtered off, then washed with ethanol again under nitrogen, then dried in a round bottom flask purged with nitrogen. A

pale brown solid product was obtained which was stored in a sealed container. Yield = 0.8g = 83%. The product was very soluble in water.

Example 170, Compound 170:

Compound 169 prepared in Example 169 was treated with 37% HCl then water was added and the entire was cooled to 0^oC at which point it was filtered to give a brown solid which was air dried overnight to give 0.35g (92% yield) title product as brown solid stable in air which had very limited water solubility.

Example 171, Compound 171:

Compound 170 from Example 170 was treated with excess Analar 25% aqueous NH_4OH which instantly dissolved the product and then was left overnight in a 50^oC oven to give quantitative conversion to a pale brown solid title ammonium salt product.

This compound was tested i.v. in mice with no toxic effect at 200 mg per kg body weight.

Example 172, Compound 172:

2.6g (0.025 mole) 3-(methylthio) propionaldehyde from Aldrich was stirred under nitrogen under reflux with 3.15g (0.025 mole) pyrogallol in 40 ml ethanol and 10 ml 37% aqueous HCl for 90 minutes following the method in Example 163 to give 3.8g purple product 72% yield ??? Compound 172a which was washed with a minimum amount of cold 0^oC ethanol, then allowed to air dry overnight. It was quantitatively converted to Compound 172b by treatment with 4 equivalents of bromine in chloroform overnight (all volatiles subsequently removed under vacuum). This dark purple product was etherified quantitatively with ethyl bromacetate following the method of Example 165 to give its ethyl acetate derivative as a pale brown heavy oil which was treated with an equal quantity of potassium hydroxide in ethanol as in Example 169 to give the title product as a yellow solid which was dried under nitrogen and stored in a sealed container.

<u>Example 173, Compound 173:</u>

Compound 172 from Example 172 was treated with HCl as in Example 170 to give virtual quantitive conversion to solid pale brown title product.

<u>Example 174, Compound 174:</u>

Compound 173 from Example 173 was treated with NH_4OH following the method in Example 171 to give quantitatively ammonium salt title product as an off-white solid.

<u>Example 175, Compound 175:</u>

The title compound was prepared as an off-white solid following synthesis of the tetramer from dodecanal and pyrogallol which was obtained as a brown solid and subsequent bromination, etherification with ethyl bromoacetate and hydrolysis with ethanolic potassium hydroxide as in Examples 163, 164, 166, and 169.

<u>Example 176, Compound 176:</u>

Compound 175 from Example 175 was treated with HCl following the method in Example 170 to give the title product as dark brown solid.

<u>Example 177, Compound 177:</u>

Compound 176 from Example 176 was treated with NH_4 OH following the method in Example 171 to give the title product as a red-brown solid.

<u>Example 178, Compound 178:</u>

The title compound was prepared as an off white solid following synthesis of the tetramer from pyrogallol and phenylacetaldehyde and conversion steps in Examples 163, 164, 166, and 169.

<u>Example 179, Compound 179:</u>

The title compound was prepared as a pale brown solid by treatment of its potassium salt from Example 178 with HCl following the method of Example 170.

Example 180, Compound 180:

The title compound was prepared as a pale yellow solid by treatment of the acid derivative from Example 179 with NH_4OH following the method Example 171.

Example 181, Compound 181:

The title compound was prepared as an off-white solid following synthesis of the tetramer from pyrogallol and m-bromobenzaldehyde and conversion steps in Examples 163, 164,166, and 169.

Example 182, Compound 182:

The title compound was prepared as a brown solid by treatment of its potassium salt from Example 181 with HCl following the method of Example 170.

Example 183, Compound 183:

The title compound was prepared as a pale brown solid by treatment of the acid derivative from Example 182 with NH_4OH following the method of Example 171.

Example 184, Compound 184:

The title compound was prepared by ommitting the bromination step in Example 181, before the etherfication step and was obtained as a pale pink solid.

Example 185, Compound 185:

The title compound was prepared as an off-white solid by treatment of its potassium salt of Example 184 with HCl following the method in Example 170.

Example 186, Compound 186:

The title compound was prepared by treatment of the acid derivative in Example 185 with thionyl chloride then bis-methoxyethyl amine following the method in Example 132 and was obtained as a pale pink solid.

Example 187, Compound 187:

The title compound was prepared as a pale yellow solid following synthesis of the yellow coloured tetramer from pyrogallol and m-nitrobenzaldehyde in 10% yield and conversion steps in Examples 163, 164,166, and 169.

Example 188, Compound 188:

The title compound was prepared as a pale brown solid by treatment of its potassium salt in Example 187 with HCl following the method of Example 170.

Example 189, Compound 189:

The title compound was prepared as a pale yellow-brown solid by treatment of its acid derivative from Example 188 with ammonium hydroxide following the method of Example 171.

Example 190, Compound 190:

The title compound was prepared as a pale brown solid following synthesis of the pale red-brown tetramer from pyrogallol and m-cyanobenzaldehyde and conversion steps in Examples 163, 164, 166 and 169.

Example 191, Compound 191:

The title compound was prepared as a pale brown solid by treatment of its potassium salt in Example 190 with HCl following the method of Example 170.

Example 192, Compound 192:

The title compound was prepared as an off-white solid by treatment of its acid derivative of Example 191 with ammonium hydroxide following the method in Example 171.

Example 193, Compound 193:

The title compound was prepared as a pale buff coloured solid following synthesis of tetramer from pyrogallol and 1-naphthaldehyde as a pale grey-pink

solid and conversion steps in Examples 163, 164, 166 and 169.

Example 194, Compound 194:

The title compound was prepared as a buff-coloured solid by treatment of its potassium salt in Example 193 with HCl following the method of Example 170.

Example 195, Compound 195:

The title compound was prepared as a buff-coloured solid by treatment of its acid derivative from Example 194 with ammonium hydroxide following the method in Example 171.

Example 196, Compound 196:

The title compound was prepared as a pale yellow solid following synthesis of the pale pink tetramer from pyrogallol and 2-naphthaldehyde and conversion steps in Examples 163, 164, 166 and 169.

Example 197, Compound 197:

The title compound was prepared as a brown solid by treatment of its potassium salt in Example 196 with HCl following the method of Example 170.

Example 198, Compound 198:

The title compound was prepared as a pale yellow-brown solid by treatment of its acid derivative from Example 197 with ammonium hydroxide following the method in Example 171.

Example 199, Compound 199:

The title compound was prepared as a bright deep orange coloured solid following synthesis of the tetramer, a purple-brown solid, from pyrogallol and 9-anthracene carboxaldehyde, and conversion steps in Examples 163, 164, 166 and 169.

Example 200, Compound 200:

The title compound was prepared as a red solid by treatment of its potassium salt of Example 199 with HCl following the method of Example 170.

Example 201, Compound 201:

The title compound was prepared as a brown solid by treatment of its acid derivative with ammonium hydroxide following the method in Example 171.

Example 202, Compound 202:

The title compound was prepared as a purple-brown solid by reacting together pyrogallol and 9-(10-chloro)anthracene carboxaldehyde to give the tetramer and thence its conversion by steps in Examples 163, 164, 166 and 169.

Example 203, Compound 203:

The title compound was prepared as a purple-brown solid by treatment of its potassium salt of Example 202 with HCl following the method in Example 170.

Example 204, Compound 204:

The title compound was prepared as a red-brown solid by treatment of its acid derivative of Example 203 with ammonium hydroxide following the method in Example 171.

Example 205, Compound 205:

The title compound was prepared as a buff solid by treatment of the tetramer from reaction of pyrogallol and pyrene carboxaldehyde, a grey-brown solid, and then its conversion in steps in Examples 163, 164, 166, and 169.

Example 206, Compound 206:

The title compound was prepared as a brown solid by treatment of its potassium salt from Example 205 with HCl following the method in Example 170.

Example 207, Compound 207:

The title compound was prepared as a brown solid by treatment of its acid derivative with ammonium hydroxide following the method in Example 171.

Example 208, Compound 208:

The title compound was prepared as a brown solid by treatment of the sulphonic acid tetramer, a pale pink solid, from reaction of pyrogallol and Compound 208a and then its conversion in steps in Examples 163, 164, 166 and 169.

Example 209, Compound 209:

The title compound was prepared as a brown solid by reaction of its potassium salt in Example 208 with HCl following the method of Example 170.

Example 210, Compound 210:

The title compound was prepared as a brown solid by reaction of its acid derivative in Example 209 with NH_4OH following the procedure in Example 171.

Example 211, Compound 211:

The title compound was prepared as a pale yellow solid by treatment of the tetramer from reaction of pyrogallol with p-bromobenzaldehyde to give a pale orange-pink solid by conversion steps in Examples 163, 164, 166 and 169.

Example 212, Compound 212:

The title compound was prepared as a pale brown solid by reaction of its potassium salt of Example 211 with HCl following the method of Example 170.

Example 213, Compound 213:

The title compound was prepared as a brown solid by reaction of its acid derivative from Example 212 with NH_4OH following the method in Example 171.

Example 214, Compound 214:

The title compound was prepared as a brown solid by treatment of the tetramer, a dark pink solid, from reaction of pyrogallol and Compound 214a and then its conversion in steps in Examples 163, 164, 166, and 169.

Example 215, Compound 215:

The title compound was prepared as a brown solid by treatment of its potassium salt derivative from Example 214 with HCl following the method in Example 170.

Example 216, Compound 216:

The title compound was prepared as a pale orange-brown solid by treatment of its acid derivative from Example 215 with NH_4OH following the method in Example 171.

Example 217, Compound 217:

The title compound was prepared as a colourless solid by firstly preparing the tetramer from pyrogallol and p-chlorobenzaldehyde and thence its conversion in steps in Examples 163, 164, 166 then 169.

Example 218, Compound 218:

The title compound was prepared as a yellow-brown solid by treatment of its potassium salt of Example 217 with HCl following the method in Example 170.

Example 219, Compound 219:

The title compound was prepared as an orange-brown solid by treatment of its acid derivative from Example 218 with NH_4OH following the method in Example 171.

Example 220, Compound 220:

The title product was obtained as a grey-blue solid by firstly preparing the tetramer from pyrogallol and p-carboxybenzaldehyde (ca only 10% yield) and

thence its conversion by steps in Examples 163, 164, 166 then 169.

Example 221, Compound 221:

The title product was obtained as a brown solid by treatment of its potassium salt from Example 220 with HCl following the method in Example 170.

Example 222, Compound 222:

The title compound was prepared as a buff solid by treatment of its acid derivative from Example 221 with NH_4OH following the method in Example 171.

Example 223, Compound 223:

Firstly pyrogallol was condensed with p-dimethyl-aminobenzaldehyde in 37% HCl:ethanol in a ratio of 1:4, refluxed for 2 hours to give a pale purple solid Compound 223a which was filtered off after addition of more ethanol and subsequently brominated with 12 equivalents of bromine in chloroform to give a bright purple-red solid Compound 223b following the method in Example 164. Etherification with ethyl bromoacetate was then carried out with 100% excess over normal quantity potassium carbonate following Example 14 in order to neutralize firstly the hydrochloride salt to the free amine derivative. The ethyl acetate derivative a red solid was then hydrolysed with ethanolic potassium hydroxide by the method in Example 169 to give the title product as an off-white solid.

Example 224, Compound 224:

The title compound was prepared as a pale brown solid by treating its potassium salt with HCl following the method in Example 170.

Example 225, Compound 225:

The title compound was prepared as a very pale brown solid by treatment of its acid derivative with NH_4OH following the method in Example 171.

Example 226, Compound 226:

Firstly pyrogallol was condensed with 2, 3, 4-trihydroxybenzene to give

the cyclic tetramer Compound 226a as a grey-brown solid which was subsequently brominated with 12 equivalents of bromine following the method in Example 164 to give a purple-brown solid product which was then etherified with 24 equivalents of ethyl bromoacetate following the method in Example 166, then treated with ethanolic potassium hydroxide following the method in Example 169 to give the title product as a pale yellow solid.

Example 227, Compound 227:

The title product was prepared as an orange solid by treatment of its respective potassium salt in Example 226 with HCl following the method in Example 170.

Example 228, Compound 228:

The title product was obtained as a brown solid by treatment of its respective acid in Example 227 with NH_4OH following the method in Example 171.

Example 229, Compound 229:

The title compound was prepared as a yellow solid by reaction of 3, 4, 5-trihydroxybenzaldehyde with pyrogallol to give the cyclic bright purple-red tetramer and similar conversion to the isomeric product in Example 226 by bromination with 12 equivalents of bromine to give a black solid, etherification with ethyl bromoacetate to give a red oil which solidified on standing and treatment with ethanolic potassium hydroxide.

Example 230, Compound 230:

The title compound was prepared as an off-white solid by treatment of its respective potassium salt with potassium hydroxide following the method in Example 170.

Example 231, Compound 231:

The title compound was prepared as a pale orange-brown solid by treatment of its acid derivative in Example 230 with ammonium hydroxide following the

method of Example 171.

Example 232, Compound 232:

The title compound was prepared as a buff coloured solid by reaction of pyrogallol with 3, 5-dibromo-4-hydroxybenzaldehyde to give an orange solid product, bromination following the method of Example 164 etherification with 32 equivalents of ethyl bromacetate following the method in Example 166, then treatment with ethanolic potassium hydroxide following the method in Example 169.

Example 233, Compound 233:

The title compound was prepared as a brown solid by treatment of its potassium salt in Example 232 with HCl following the method in Example 170.

Example 234, Compound 234:

The title compound was prepared as a pale red-brown solid by treatment of its acid derivative with NH_4OH following the method in Example 171.

Example 235, Compound 235:

The title compared was prepared as a very pale-brown solid by reaction of pyrogallol with 3, 4, 5-trimethoxybenzaldehyde to give a brown solid, bromination with 12 equivalents of bromine following the method in Example 164, to give a pale pink orange solid, etherification with ethyl bromoacetate following the method in Example 166, then treatment with ethanolic potassium hydroxide following the method in Example 169.

Example 236, Compound 236:

The title compound was prepared as a brown solid by treatment of its potassium salt of Example 235 with HCl following the method in Example 170.

Example 237, Compound 237:

The title compound was prepared as a pale red-brown solid by treatment of its acid derivative from Example 236 with NH_4OH following Example 171.

Example 238: P-T-Butylcalix-4-arene Tetraacetic Acid

The compound was prepared as a colourless solid from its ethyl acetate derivative following the method in Example 12 after filtration and washing with ice cold water and allowing to air-dry overnight. I.r.ν1730 S C=Ocm^{-1}.

Example 239: P-T-Butylcalix-6-arene Hexaacetic Acid

The title compound was prepared as a colourless solid following the method in U.S. Patent No. 5,132,345 by S. J. Harris, M. A. McKervey, G. Svehla and D. Diamond, July 21 1992 except that ethanol alone was used in place of ethanol/water mixture in which KOH was dissolved with the same work up as the method in Example 238. I.r.ν1730 S C=Ocm^{-1}.

Example 240: P-Ethylcalix-7-arene Heptaacetic Acid

The title compound was prepared as a colourless solid from its ethylacetate derivative following the method in Example 239. I.r.ν1730 S C=Ocm^{-1}.

Example 241: P-T-Butylcalix-8-arene Octaacetic Acid

The title compound was prepared as a colourless solid by hydrolysis of its ethyl acetate derivative, prepared following the method in U.S. Patent No. 4,556,700 by S. J. Harris, M.A. Kervey, D. P. Melody, J. Woods and J. M. Rooney, December 3rd 1985, and J. Am Chem. Soc. III p.868, 1989 by F. Arnand-Neu, E. M. Collins, M. Deasy, G. Ferguson, S. J. Harris, B. Kaitner, A. J. Lough, M. A. McKervey, E. Marques, B. Ruhl, M. J. Schwing-Weill and E. M. Seward with ethanolic KOH following the method in Example 12. I.r.ν1730 S C=Ocm^{-1}.

Example 242: P-T-Butylcalix-4-arene Tetra-potassium Acetate

The title compound was prepared as a colourless solid following the method in Example 12 prior to neutralisation step by simply filtering off the product from cooled ethanolic reaction mixture and washing with a minimum quantity of ice-cold ethanol. I.r.ν1610 S C=Ocm^{-1}.

Example 243: P-T-Butylcalix-6-arene Hepta-potassium Acetate

The title compound was prepared as a colourless solid following the method in Example 239 and 242. I.r. ν 1610 S C=Ocm^{-1}.

Example 244: P-Ethylcalix-7-arene Hepta-potassium Acetate

The title compound was prepared as a colourless solid following the method in Example 240 and 242. I.r. ν 1610 S C=Ocm^{-1}.

Example 245: P-T-Butylcalix-8-arene Octa-potassium Acetate

The title compound was prepared as a colourless solid following the method in Example 241 and 242. I.r. ν 1610 S C=Ocm^{-1}.

Example 246: P-T-Butylcalix-4-arene Tetra-diethylthioacetamide Derivative

The title compound was prepared as a pale yellow solid following the method in European Patent Application No. 90313382.5 by S. J. Harris, J. Guthrie, E. M. Collins, C. McArdle and M. A. McKervey, December 13 1989.

Example 248: P-T-Butylcalix-4-arene Tetra-diethylacetamide Derivative

The title compound was prepared as a colourless solid following the method in reference cited in Example 246.

Example 249, Compound 249:

This compound was prepared following the method cited in Example 246.

Example 250: P-T-Butylcalix-4-arene Tetra-ethyl Acetate Derivative

The title compound was prepared as a colourless solid following the method in U.S. Patent 4,556,700 by S. J. Harris, M.A. McKervey, D. P. Melody, J. Woods and J. M. Rooney, December 3rd 1985.

Example 251, Compound 251:

To 3.1g of p-t-butylcalix-4-arene acetyl chloride (0.0032 mole) from

Example 12 in 20 mls NaH dried tetrahydrofuran at room temperature was added 2.38g (0.0128 mole) 1-dodecanol and 2.04g (0.026 mole) dry pyridine under nitrogen with stirring. The reaction mixture was stirred at RT for 24 hours then poured into water. A colourless sticky solid formed which was washed well with water then dried to give the title product as a colourless heavy oil which solidified on standing to give a colourless waxy solid mp 38-9^{0}C. I.r.ν1755 S C=0cm^{-1}.

Example 252: P-Bromocalix-4-arene Tetra-ethyl Acetate Derivative

The title compound was prepared as a colourless crystalline solid as described in Example 37.

Example 253: P-T-Butycalix-4-arene Diethyl Acetate

The title compound was prepared as a colourless solid following the procedure in U.S. Patent No. 4,642,362 by S. J. Harris, J. G. Woods and J. M. Rooney, February 10th 1987.

Example 254: P-T-Butylcalix-6-arene Hexa-ethyl Acetate Derivative

The title compound was prepared as a colourless crystalline solid following the method cited in Example 241.

Example 255: P-T-Butylcalix-8-arene Octa-ethyl Acetate Derivative

The title compound was prepared as a colourless crystalline solid following the method cited in Example 241.

Example 256: P-T-Butyldihomooxacalix-4-arene Tetra-acetic Acid

The title compound was prepared as a colourless solid following the method cited in Example 53.

Example 257: P-T-Butycalix-4-arene Tetra-t-butylketone

The title compound was prepared as a colourless solid following the method cited in U.S. Patent No. 5,132,345 by S. J. Harris, M. A. McKervey, G. Svehla and D. Diamond, July 21st 1992.

Example 258, Compound 258:

To 1.62g (0.025 mole) p-t-butycalix-4-arene was added 3.9g (0.0103 mole) tetrabutylammonium iodide and 1.42g (0.0103 mole) anhydrous potassium carbonate and 0.91g (0.010 mole) chloroacetone in 50 mls andar acetone and the entire was refluxed under nitrogen for 17 hours, following which the volatiles were removed and the residue was taken up into dichloromethane which was washed well with water to give after drying of the organic layer with dried magnesium sulphate 1.8g (94% yield) title product as a colourless solid mp 121-2^{o}C I.r.ν 3400M OH 1720 S C=Ocm^{-1}. Elemental analysis calculated for $C_{50}H_{64}O_6.1/2CH_2Cl_2$ C=75.48, H=8.15, Found C=76.16, H=8.84%.

Example 259, Compound 259:

The title compound was prepared following the procedure given in U.K. Patent Application No. 2,200,909 by S. J. Harris, August 17th 1988.

Example 260, Compound 260:

The title compound was prepared as a colourless solid from p-t-butylcalix-4-arene tetra-acetic acid following the method cited in Example 246 employing di-n-didodecylamine in place of diethylamine.

Example 261, Compound 261:

The title compound was prepared as a colourless solid following the method cited in Example 246.

Example 262, Compound 262:

4.9g (0.0051 mole) Compound 262a prepared following the procedure cited in Example 12 was dissolved in 35 mls of dry tetrahydroforan which was added dropwise under nitrogen to 30 mls dry THF containing 4g (0.031 mole) methyl prolinate and 4.2 mls (0.0306 mole) triethylamine with stirring at room temperature. The resulting mixture containing much white precipitate was then stirred under reflux for a further 6 hours. The cooled reaction mixture was then filtered through celite to remove inorganic salts and volatiles removed the last traces under reduced pressure to give 10.0g crude product which was recrystallised from dichloromethane/methanol to give 5.1g 49% yield of

colourless crystals of the title product mp 243-5^{O}C. Elemental analysis $C_{76}H_{100}N_4O_{16}$ requires C=68.88, H=7.55, N=4.23, Found C=68.60, H=7.74, N=3.92%.

<u>Example 263, Compound 263:</u>

The title compound was prepared as a soluble linear polymer following the method cited in European Patent Application No. 0,309,291 by S. J. Harris, M. G. MacManus and J. Guthrie, 29th March 1989 .

<u>Example 264, Compound 264:</u>

The title compound was prepared as a colourless solid following the method in U.S. Patent No. 4,882,449 by S. J. Harris November 21st 1989.

<u>Example 265, Compound 265:</u>

The title compound was prepared following the method cited in European Patent Application No. 90313382.5 by S. J. Harris, J. Guthrie, M. MacManus, C. McArdle and M. A. McKervey, December 13 1989.

<u>Example 266, Compound 266:</u>

The title compound was prepared following the method cited in Example 263.

<u>Example 267, Compound 267:</u>

The title compound was prepared as a colourless solid following the method cited in Example 263.

<u>Example 268, Compound 268:</u>

The title compound was prepared following the method cited in U.S. Patent No. 4,957,960 by S. J. Harris, J. G. Woods, J. M. Rooney, M. MacManus and J. Guthrie, September 18th 1990.

<u>Example 269, Compound 269:</u>

The title compound was prepared as a colourless solid by treatment of

cone calix-4-arene t-butylacetate derivative with ethanolic KOH thence HCl following the method in Example 12.

Example 270, Cone Compound 270:

P-Ethylcalix-7-arene heptaacetic Acid prepared in Example 240 was treated with thionyl chloride as in Example 12 to give its acetyl chloride then bis(hydroxyethyl)amine following the method cited in Example 246 to give the title product.

Example 271, Compound 271:

The title product was prepared by treating p-t-butylcalix-4-arene tetra-acetyl chloride with di-n-octylamine and triethylamine following the method cited in Example 246/8.

Example 272, Compound 272:

The title compound was prepared as in Example 271 utilising bis(methoxethyl)amine in place of dioctylamine.

Example 273, Compound 273:

P-Bromodihomooxacalix-4-arene tetra-acetic acid prepared in Example 70 was treated with thionyl chloride then di-n-octylamine following the method cited in Example 271 to give the title product.

Example 274, Compound 274:

The title compound was prepared following the method of Example 138 bis(methoxyethyl)amine being used in place of bis(hydroxyethyl)amine.

Example 275, Compound 275:

The title compound was prepared following the method cited in Example 271 substituting di-n-decylamine for di-n-octylamine.

Example 276, Compound 276:

Following the method cited in Example 138 the tetraethyl acetate derivative of p-t-butyldioxacalix-4-arene was hydrolysed with ethanolic KOH to give upon acidification the tetraacid derivative as a colourless solid. I.r. 3370 S broad OH, 1735 S C=Ocm^{-1}. This acid was converted into its acid chloride by 2 hours reflux in thionyl chloride under nitrogen to give after removal of all volatiles acid chloride product as a buff solid. I.r. 1810 S C=Ocm^{-1}. To 3.5g (0.0034 mole) of the tetraacid chloride was added 20 mls of dry THF then at $0^{o}C$ dropwise under nitrogen was added 2.98g (0.041 mole) diethylamine. A white precipitate formed. The reaction mixture was allowed to stir for 17 hours at room temperature following which all volatiles were removed and the residue was taken up in 25 mls dichloromethane which was washed twice with water then dried over dried magnesium sulphate to give after removal of solvent 3.8g 90% yield of crude title product as a pale pink solid. Chromatography on neutral alumina using dichloromethane as eluent gave 2.1g high purity very pale pink solid mp 52-5^{o}C. I.r.ν 1643 S C=Ocm^{-1}. Elemental analysis calculated for $C_{70}H_{104}O_{10}N_4$-CH_2CL_2 C=68.41, H=8.57, O=12.83, N=4.49, Found C=69.17, H=9.10, O=12.42, N=4.57%.

Example 277, Compound 277:

P-T-Butylcalix-5-arene was prepared following the method cited in Example 3 then etherified with ethyl bromoacetate following the method cited in Example 14 to give its pentaethyl acetate derivative which was then converted to its acid salt, acid, acid chloride thence amide following the method cited in Example 246.

Example 278, Compound 278:

0.480g (0.001 mole) G-orange (Fluta) (compound 278a) was reacted with 0.126g (0.001 mole) pyrogallol in refluxing 37% aqueous HCl to ethanol under nitrogen following the method in Example 163 to give 0.245g 50% title product as a deep red-orange solid following washing with minimum quantity methanol.

Example 279, Compound 279:

Treatment of Compound 278 from Example 278 with 32 equivalents ethyl bromoacetate and 36 equivalents K_2CO_3 in refluxing dry acetone for 48

hours following the method of Example 14 gave the title product as a pale brown oil.

Example 280, Compound 280:

Compound 163 from Example 163 was quantitatively converted to its chloro derivative compound 280a by bubbling chlorine gas into a chloroform solution of same for 2 hr at room temperature and thence removal of all volatiles then converted to the title product, following the method in Example 166 and 167, as a pale yellow solid.

Example 281, Compound 281:

Compound 163 from Example 163 was treated with 4 equivalents ICl in chloroform overnight to give quantitative conversion to is iodo derivative compound 281a following removal of volatiles, then converted to the title compound, following the method in Example 166 and 167, as a pale pink solid.

Example 282, Compound 282:

0.238g (0.00025 mole) of compound 282a prepared following the procedure in Example 12 was dissolved in 5mls dry tetrahydrofuran which was added dropwise under nitrogen to 5mls dry THF containing 0.462g (0.001 mole) cholesterylaniline (Sigma) and 0.107g (0.0013 mole) pyridine, in the dark with stirring at room temperature for 48 hours. This was followed by removal of all volatiles under vacuum and treatment of residue (off white solid) with water and air drying at room temperature overnight to give 0.540g title product as an off-white solid. Yield 82%. I.r. ν 1670 S C=0 cm^{-1}

Antiviral Assays

The anti-HIV and anti-SIV (Simian immunodeficiency virus) activities and toxicities of compounds were assessed in C8166 cells infected with $HIV\text{-}1_{111B}$, $HIV\text{-}2_{ROD}$ or SIV_{MAC}. The cells are cultured in RPM1 1640 with 10% calf serum.

Aliquot of 4×10^4 cells per microtiter plate well were mixed with 5 fold dilutions of compounds prior to addition of 10 $CCID_{50}$ units of virus and incubated for 5-6 days. Formation of syncytia was examined from 2 days post-infection. Gp120 antigen produced at 5-6 days was measured by ELISA,

using the lectin GNA (from Galanthus nivalis) to capture the glycoprotein and human anti-HIV serum for detection (11). Cell viability of infected, and uninfected control cells was measured by the MTT-Formazan method (12).

Briefly, 10 µl of a solution of 3-(4,5-dimethylthiazol-2-YL)-2,5 diphenyl-tetrazolium bromide (MTT, 7.5 mg/ml in PBS) was added to each well containing 100 ul of infected or uninfected cells. After incubating at 37°C for 1 hr, the blue formazan crystals produced are solubilized in 150 ul of 10% V/V triton X-100 in acidified isopropanol (2 ml concentrated HCl per 500 ml solvent) and absorbance read at 540nm.

gp 120 Antigen Assay

A microtiter antigen capture ELISA was developed using lectin (GNA) from Galanthus nivalis (Vector Laboratories, Peterborough, U.K.) and human antibodies (12). The plates were coated with lectin (0.5ug), and after blocking with 10% calf serum, dilutions of virus supernatant in 0.25% detergent solution (Empigen, Albright and Wilson Ltd., Whitehaven, U.K.) were added to the wells and incubated at 4°C for 12-16 hours. Bound antigen was captured using human anti-HIV antibodies, and finally detected with anti-human Ig antibodies conjugated to horseradish peroxidase. The results are shown in Table 1.

Results

Table 2 shows the effect of the compound of Example 169 on viral infectivity. Virus was incubated at room temperature with dilutions of the compound to be tested, and the compound was then removed by dilution to a virus non-inhibiting concentration. The viral infectivity was determined by adding C8166 cells and compared with untreated controls. The compound did not reduce the virus titre indicating:-

(a) the compound does not inhibit infection by binding to viral envelope proteins, or
(b) the binding was reversible, or
(c) the inhibition of fusion (of viral and cell envelopes after binding) is inhibiting infection.

Table 3 describes the results of preliminary experiments to determine the

mode of action of the compounds of the present invention. Antiviral activity is pronounced for the compounds tested when the compound is present during virus absorption, then when added 4 hours after infection suggesting that the compound of Example 169 is inhibiting infection at an early stage of virus replication (e.g. virus absorption, fusion, uncoating or reverse transcriptase activity), similarly to heparin.

The compound was tested for inhibition of reverse transcriptase in vitro but was found to have no effect on it's activity.

The compounds tested appear to be highly active against HIV-1 and less so against HIV-2 and SIV.

The compounds of Example 169 and 171 did not induce any signs of toxicity as shown by observation of behaviour over 5 days, in groups of three male and three female mice, after intravenous administration at 50mg/kg. Similarly there was no apparent toxicity at a dose of 200mg/kg following intravenous injection in groups of 2 male mice.

Table 4 shows how the determination of EC_{50} and TC_{50} was made for the compounds shown in Tables 5 to 13. The results for AZT are shown as a control.

Table 1

Compound	EC_{50}µm	EC_{50}µm	EC_{50}µm
HIV-2	SIV	HIV-1	
Example 169			
20-40	100	0.032	
Example 171			
10	20	0.03	
Heparin	0.8	1	-

EC_{50} represents the concentration which reduces the Ag gp120 by 50% in infected cell cultures.
TC_{50} represents the concentration of drug which reduces cell growth by 50%.

The compound of Examples 169 and 171 appear to be selective for HIV -<u>1</u> not HIV -<u>2</u> nor SIV.

Table 2: Effect of compounds on viral infectivity

Compound	Conc.[1] (μg/ml)	Viral titre[2]
DS (5000mw)	100	8000
Plant Ext.k	10	<1
Myrecetin	10	128
Example 169	5	8000
Control		8000

[1] Virus (10^5-10^6 $TCID_{50}$) was incubated with the compounds at the concentrations indicated for 60 minutes at room temperature.
[2] Viral titre was determined by serially diluting the compound/virus mixture before mixing with C8166 cells. The end point was measured by examining syncytia and by the MTT Formazan assay after five days of infection.

Table 3: Effect of the Time of Addition of Compounds

Compound	Conc μM	Cell Viability 0 hr	Cell Viability 4 hr	Antigen gp120 % Control 0 hr	Antigen gp120 % Control 4 hr	EC_{50} μM 0 hr	EC_{50} μM 4 hr
Example 169	10	98	33		48	0.2	10
	2	78	24		100		
	0.4	31	22	23			
	0.08	24	22	63			
Heparin	100	100	31		52	2	100
	20	58	27	17	79		
	4	33	23	47	100		
	0.8	25	22	98			
AZT	2	99	100			0.006	0.016
	0.4	98	66	16	19		
	0.08	53	32	22	36		
	0.016	35	27	44	54		
	0.003	25	24	84	96		

0hr= cells were mixed with the dilutions of compounds just before adding virus

4hr= cells were incubated at 37^{0} with virus for 4 hours prior to the addition of compounds.

EC_{50} represents the concentration which reduced the Ag gp120 by 50% in infected cell cultures.

Reversibility is indicated for Compound Example 169.

Table 4: Anti-HIV Activity

Compound	Conc (μM)	Syncytia (+/-)	Ag gp120 % of Control	Est. Cell Growth % Control Infected	Uninfected	EC_{50} μM	TC_{50} μM
EXAMPLE 171	500	-		87	87	0.03	>500
	50	-		109	101		
	10	-		103	104		
	2	-/+		87	102		
	0.4	+/-	17	65	99		
	0.08	+	43	41	107		
	0.016	+	70	30	107		
AZT	10	-		99	96	0.016	>1000
	2	-/+		85	97		
	0.4	-/+		78	99		
	0.08	+/-	27	56	96		
	0.016	+	53	26	98		
	0.0032	+	82	24	97		
Control		+	100	24			

Virus = HIV1 III B

Cells = C8166

EC_{50} is the concentration which reduces the Ag gp120 by 50% in infected cell cultures.
TC_{50} is the concentration of drug which reduces cell growth by 50%.

Table 5: Parent (Phenolic) Calixarenes (and Oxacalixarenes)

Example	EC_{50} μM	TC_{50} μM	TC_{50}/EC_{50}
1	0.8	5	6.3
2	25	150	6
3	0.8	5	6.3
4	50	100	2
5	20	>100	>5
6	Inactive	>500	-
7	200	>250	>1.25
8	20	>500	>25
9	50	250	5
10	20	500	25
11	20	20	1
AZT	0.016	>1000	>62,500

Table 6: Ester Modified (Oxa) Calixarenes

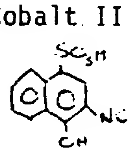
Example	EC_{50} µM	TC_{50} µM	TC_{50}/EC_{50}
12*	4	10	2.5
13*	0.8	100	125
14	250	>1250	>5
15	100-200	500	2.5-5
16*	0.4	1000	2500
17	33	>200	>6
18	5	15	3
19	50	>100	>2
20	4	40	10
21	20	150	7.5
22	5	150	30
23	Inactive	40	-
24	50	50	1
25	20	50	2.5
26	50	>100	>2
27	50	50	1
28	Inactive	50	-
29	Inactive	-	-
30	9	>13	>1.4
31	Inactive	-	-
32	19	130	7
33	Inactive	-	-
34	Inactive	-	-
35	Inactive	-	-
HO CH_2 $CH_2P(O)(OCH_3)_2$*	2500	>5000	>2
AZT	0.016	>1000	>62,500

*Calixarene and oxacalixarene derivatives of $HOCH_2CH_2P(O)(OCH_3)_2$

Table 7: Acid/Salt Modified (Oxa) Calixarenes

Example	EC_{50} µM	TC_{50} µM	TC_{50}/EC_{50}
36	250	>500	>2
37	10	>100	>10
38	10	>100	>10
39	0.8	>50	>62.5
40	0.4	150	375
41	50	200	4
42	200	>1000	>5
43	0.16	500	3125
44	Inactive	>1000	-
45	50	>100	>2
46	200	1000	5
47	0.5	>5000	>10,000
48	0.2	>1250	>6250
49	0.6	500-800	833-1333
50	8	>5000	625
51	0.4	>500	>1250
52	20	200	10
53	8	50	6.3
54	5	50	10
55	2	50	25
56	4	50	12.5
57	10	150	15
AZT	0.016	>1000	>62,500

Table 7 Contd/....

	EC_{50} μM	TC_{50} μM	TC_{50}/EC_{50}
58	4	10	2.5
59	5	150	30
60	5	15	3
61	5	10	2
62**	Inactive	25	-
63	20	20	1
64	15	25	1.7
65	2	50	25
66	2	50	25
67	Inactive	-	-
68	2	100	50
69	5	300	60
70	20	>250	12.5
71	15	50	8.3
72	8	300	37.5
73	20	>100	>5
74	10	>100	>10
75	5	>100	>20
76	2-4	250	62.5-125
77	10	>100	>10
78	0.8	400	500
79	4	250	62.5
80	2	>100	>50
81	2	20	10
82	10	>100	>10
83	8	>100	>12.5
Cobalt II Complexer	200	>5000	25
 (Fluka)			
AZT	0.016	>1000	62,500

** = a cobalt II salt of an oxacalixarene

Table 7 Contd/....

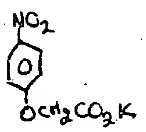
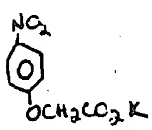
84	0.8	>100	>125
85	2	100	50
86	0.25	2000-2500	8000-10000
87	50	>100	>2
88	1-2	250	125-250
89	0.8	>100	>125
90	0.8	20	25
91	50	>50	>1
92	4	50	12.5
93	100	>100	>1
94	10	>100	>10
95	1	50	50
	2000	2000	1
 (Fluka)	2500	>5000	>2
AZT	0.016	>1000	>62,500

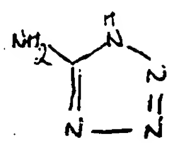
Table 8: Open Chain Phenol Formaldehyde Oligomers and Derivatives (Tetramers)

Example	EC_{50} μM	TC_{50} μM	TC_{50}/EC_{50}
96	20	300	15
97	20	100	5
98	8	300	37.5
99	20-40	>200	>10
100	0.64	>200	>313
101	2	>200	>100
AZT	0.016	>1000	>62,500

Table 9: Amide Modified (Oxa) Calixarenes

Example	EC_{50}	TC_{50}	TC_{50}/EC_{50}
102	20	50	2.5
103***	50	50	1
104	Inactive	-	-
105	2.4	3.7	1.5
106	200	500	2.5
107	50	200	4
108	10	50	5
109	100	>500	>5
110	50	250-500	5-10
111	100	500	5
112	3.9	7	1.8
113	20	250	12.5
114	40	>100	>2.5
115	50	750	15
116	20	1000	50
117	25	250-500	10-20
118	20	500	25
119	2-4	100	25-50
120	20	100	5
121	100	>500	>5
122	40	>500	>12.5
123	40	>500	>12.5
124	200	>500	>2.5
125	250	500	2
126	40	400	10
127	40	>500	>12.5
128	50	250	5
129	40	>500	>12.5
130	4	16	4

Table 9 Contd/....

131***	40	200	50
132	40	1000	25
133***	0.25	1000	4000
134	>10	10-20	1 approx.
135	10	80	8
136	87	>100	>1.2
137	10	100	10
138	6.6	24	3.6
*** 	Inactive	2000	-

*** = an (oxa)calixarene derivative of aminotetrazole

Table 10: Antibiotic Modified (Oxa) Calixarenes

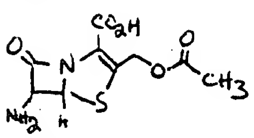
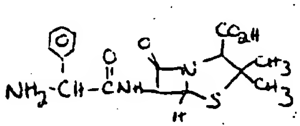
Example	EC_{50} μM	TC_{50} μM	TC_{50}/EC_{50}
		Polyene Macrocyle	
139	0.64	100	156
141	0.8	15-20	18.8-25
AmphotericinB (Sigma)	100	>250	>2.5
143	4	>25	>6.3
Nystatin A (Sigma)	100	>500	>5
142	20	30	1.5
		Lactam	
140	10	50	5
144	2	>50	>25
148	8	50	6.3
149	2	50	25
 aminocephalosporanic acid (Fluka)	50	80	1.6
145	20	250	12.5
 Ampicillin	500	>2500	>5
146	10-20	200-300	10-30
		Aminoglycoside	
147	10	200	20
AZT	0.016	>1000	>62,500

Table 11: Cycloveratrylene Acid & Acid Salt Derivatives

Example	EC_{50} µM	TC_{50} µM	TC_{50}/EC_{50}
150	0.8	500-1000	625-1250
151	1.6	500	313
152	0.5	500	1000
AZT	0.016	>1000	>62,500

Table 12: Hogberg Compounds and Derivatives

Example	EC_{50} μM	TC_{50} μM	TC_{50}/EC_{50}
153	20	125	6.3
154	10	125	12.5
155	20	250	12.5
156	25	2500	100
157	5-10	5000	500-1000
158	16	200	12.5
159	1.6	>200	>125
160	1.6	>200	>125
161	100	>200	>2
162	100	>200	>2
AZT	0.016	>1000	>62,500

Table 13: Pyrogallol-Aldehyde Cyclic Tetramers and Derivatives

Example	EC_{50} µM	TC_{50} µM	TC_{50}/EC_{50}
163	5	125	25
164	50	50	1
165	100	>500	>5
166	8-16	100	6.3-12.5
167	200	>5000	>25
168	10-20	>5000	>250
169	0.032	>200	>6250
170	0.02	160	8000
171	0.03	>500	>16,666
172	20	>1000	>50
173	20	500	25
174	400	>1000	>2.5
175	0.32	800-1000	2500-3125
176	0.32	200-400	625-1250
177	0.128	800-1000	6250-7813
178	0.16	>1000	>6250
179	0.16	>500	>3125
180	0.10-0.16	>1000	>6250
181	0.05-0.06	>1000	>16,667
182	0.10-0.20	100-200	500-2000
183	0.02-0.05	1000	20,000-50,000
184	4	>1000	>250
185	4	>500	>125
AZT	0.016	>1000	>62,500

Table 13 Contd/....

	$EC_{50}\mu M$	$TC_{50}\mu M$	TC_{50}/EC_{50}
186	4	20	5
187	0.8	1000	1250
188	0.4-0.8	500-1000	625-2500
189	1-2	500-1000	250-1000
190	2-4	>1000	>250
191	0.4-0.8	500	625-1250
192	0.4	500-1000	1250-2500
193	0.64	>2000	>3125
194	0.16	500	3125
195	0.32	>2000	>6250
196	0.08-0.16	>1000	>6250
197	0.02-0.06	800	13,332-40,000
198	0.064	1000	15,624
199	0.16	>1000	>6250
200	0.02	50	2500
201	0.016	250	15,625
202	0.32	200-400	625-1250
203	0.20	20	100
204	0.08	50-100	625-1250
205	0.5-1.0	500	500-1000
206	0.8	250	313
207	0.32	50	1563
208	80	>1000	>12.5
209	20	>50	>2.5
210	>250	>250	1 approx.
211	0.8	>2000	>2500
212	0.16	1000	6250
213	0.32	>2000	>6250
214	0.16-0.32	>2000	>6250
215	0.2	2000	10,000
216	0.1-0.2	2000	10,000-20,000
AZT	0.016	>1000	>62,500

Table 13 Contd/....

217	0.16	>1000	>6250
218	0.10-0.16	>1000	>6250
219	0.10-0.16	>1000	>6250
220	0.2	>1000	>5000
221	0.2	>250	>1250
222	0.2	>1000	>5000
223	0.16	500	3125
224	10-20	>1000	>50
225	10-20	>1000	>50
226	2	500	250
227	4	200	50
228	10	50	5
229	20	>500	>25
230	20-40	100-200	2.5-10
231	100	500	5
232	1.6	40	25
233	0.4	50-100	125-250
234	0.6	200	333
235	1.6	>1000	>625
236	3.2	1000	313
237	0.32	1000	3125
278	0.015	100	6666
279	0.08	250	3125
280	0.08	500	6250
281	0.064	>1000	>15,625
AZT	0.016	>1000	62,500

Antifungal Activity

Cultures of the following wood fungi were obtained from the collections stated.

1.	Fusarium oxysporum	ATCC 10960
2.	Coriolous versicolor	CMI 79126
3.	Trichophyton rubrum	ATCC 28188
4.	Fusarium graminearum	CMI 154209
5.	Pyricularia versicolor	CMI 146890
6.	Trichophyton mentagarophytes	ATCC 4807

ATCC = American Type Culture Collection
CMI = Commonwealth Mycological Institute

Fusarium oxysporum, Fusarium graminearum, Pyricularia versicolor and coriolous versicolor were all grown on (Oxoid) potato dextrose agar. Trichophyton mentagarophytes was grown on potato dextrose agar with 0.5% yeast extract (Difco) added. Trichophyton rubrum was grown on (Oxoid) szabaraud dextrose agar.

Sample compounds and bis (tributyltin oxide) were prepared in acetone and $CuSO_4$ in water. From the data obtained by Yagi et al (13) it was decided to look at concentrations of 10, 100 and 1,000 μM samples in the case of bis (tributyltin oxide) and $CuSO_4$ and concentrations of 1, 10 and 100 μM samples in the case of the test compound were tested.

The appropriate growth media were prepared and autoclaved in 100 ml lots. These were then cooled to 50^{o}C and 1 ml of respective samples at concn. x 100 was added, i.e. for concn. of 1,000 μM, 1 ml of 100 mM sample solution was added.

Controls were set up for each fungus, one set with no addition, and one set with 1 ml of acetone added. All tests were carried out in quadruplicate.

4 plates were poured from each flask and allowed to set. Discs of fungi, 8 mm in diameter, from well grown plates were applied to the test plates, and incubated at 25^{o}C.

After a suitable incubation time (4-10 days, depending on rate of growth of individual fungi) growth diameters were measured on all plates and the appropriate data is shown in Table 14. Bis (tributyltin oxide) is a potent inhibitor of all 6 fungi tested, it totally inhibited growth of 5 fungi at concentrations of 10, 100 and 1,000 μM (Table 1; discs, 8mm in diameter, of fungi were applied to the test plates and therefore a growth diameter of 8 mm implies no growth of the organism on the test plate). <u>Fusarium graminearum</u> CMI 154209 was the only organism capable of growing in the presence of this compound and the ED_{50} value for this organism was exceptionally low, i.e. 3.6×10^{-2} μM.

Table 14: Antifungal Activity

Organism	(Aqueous) $CuSO_4$ ED_{50} (μM)	(Acetone) Bis(Tributyltin oxide) ED_{50} (μM)	Example 238	Example 239
Fusarium oxysporum ATCC 10960 (5d)	no inhibition	total inhibition	$1.2x10^4$	$7.9x10^4$
Fusarium graminearum CMI 154209 (7d)	$2.1x10^7$	$3.6x10^{-2}$	$9.84x10^2$	$7.4x10^{12}$
Trichophyton rubrum ATCC 28188 (7d)	$7.1x10^5$	total inhibition	$5.79x10^3$	$1.3x10^6$
Trichophyton mentagarophytes ATCC 4807 (7d)	no inhibition	total inhibition	$2.7x10^4$	$7.3x10^4$

Contd/....

Table 14: Antifungal Activity Continued

Organism	(Aqueous) $CuSO_4$ ED_{50} (μM)	(Acetone) Bis(Tributyltin oxide) ED_{50} (μM)	Example 238	Example 239
Pyricularia versicolor CMI 146890 (7d)	$5.4x10^2$	total inhibition	$4x10^4$	$7.8x10^{14}$
Coriolous versicolor CMI 79126 (5d)	$1.9x10^3$	total inhibition	$2.9x10^5$	no inhibition

ND = Not determined

Table 14: Antifungal Activity

Organism	Example 240	Example 241	Example 242	Example 243
Fusarium oxysporum ATCC 10960 (5d)	$1.42x10^4$	$4.75x10^4$	10^{12}	no inhibition
Fusarium graminearum CMI 154209 (7d)	$2.9x10^5$	$7.65x10^3$	$3.7x10^5$	$2.6x10^5$
Trichophyton rubrum ATCC 28188 (7d)	no inhibition	$4.1x10^3$	$2.2x10^6$	no inhibition
Trichophyton mentagarophytes ATCC 4807 (7d)	no inhibition	$7.7x10^3$	$1.3x10^8$	$1.36x10^2$
Pyricularia versicolor CMI 146890 (7d)	no inhibition	$4.9x10^2$	$4.4x10^3$	$1.95x10^2$

Contd/....

Table 14: Antifungal Activity Continued

	Example 240	Example 241	Example 242	Example 243
Coriolous versicolor CMI 79126 (5d)	no inhibition	3.9×10^2	no inhibition	2.3×10^3

ND = Not determined

Table 14: Antifungal Activity

Organism	Example 244	Example 245	Example 246	Example 247
Fusarium oxysporum ATCC 10960 (5d)	no inhibition	4.3×10^{14}	1.0×10^{17}	9.4×10^{9}
Fusarium graminearum CMI 154209 (7d)	no inhibition	7.5×10^{11}	4.52×10^{2}	1.8×10^{6}
Trichophyton rubrum ATCC 28188 (7d)	1.7×10^{8}	no inhibition	2.78×10^{3}	5.2×10^{5}
Trichophyton mentagarophytes ATCC 4807 (7d)	3.8×10^{7}	7.1×10^{17}	1×10^{-3}	no inhibition
Pyricularia versicolor CMI 146890 (7d)	none	10.5×10^{5}	4.4×10^{5}	no inhibition

Contd/....

Table 14: Antifungal Activity Continued

	Example 244	Example 245	Example 246	Example 247
Coriolous versicolor CMI 79126 (5d)	none	9.9×10^7	7.6×10^5	no inhibition

ND = Not determined

Table 14: Antifungal Activity

Organism	Example 248	Example 249
Fusarium oxysporum ATCC 10960 (5d)	1.23×10^2	8.7×10^9
Fusarium graminearum CMI 154209 (7d)	6.56×10^2	1.4×10^3
Trichophyton rubrum ATCC 28188 (7d)	2.3×10^4	4.9×10^4
Trichophyton mentagarophytes ATCC 4807 (7d)	3.9×10^3	8.68×10^2
Pyricularia versicolor CMI 146890 (7d)	4.7×10	3.5×10^3

Contd/...

Table 14: Antifungal Activity Continued

	Example 248	Example 249
Coriolous versicolor CMI 79126 (5d)	3.33×10^2	no inhibition

ND = Not determined

Activity against C.albicans

Samples were dissolved in sterile water, and diluted again in sterile water as below.2ml aliquots of each sample was added to petri dishes, 18 ml of molten SD agar was then added to each plate, producing a 1/10 dilution of sample.

When plates were set and dry, they were inoculated with 10 microlitre applications of **Candida albicans** suspension in the order of $1x10^6$ cells/ml. This suspension was produced by touching an overnight colony with a loop and inoculating 2mls of sterile water, using a Hemocetometer and a microscope at X 40.

The plates were incubated at 30^{0}C for 20 hours.

SAMPLE	500 g/ml	300 g/ml	100 g/ml
Example 40	X	---	---
Example 169	X	X	---

X = indicates inhibition of Candida albicans.

Antimycotic Activity

The in vitro antimycotic activity of tested compounds was determined against a series of yeast and fungi and has been evaluated through the minimum inhibitory concentration (MIC) according to the method of progressive double dilutions in liquid Casitone medium (A).

The yeasts were originally clinical isolates and were typed by conventional methods (B, C). The cells were maintained by a periodic subculture on malt agar (Oxoid) slants. MIC determinations were performed by a microtitre technique on freshly subcultured two day old cells from slants. One (1mm) loopful of the freshly cultured cells was suspended in sterile distilled water (10 ml). The seeding rate was adjusted by successive dilution of this stock solution with sterile distilled water until the optical density reading of the solution was 0.05 (530 nm) (D, E). 25µl of this diluted cell suspension was then added to the wells in the autotray microtitre plate

already containing liquid casitone medium (Difco; 20%, 0.1 ml).

The compounds were then added to the wells in the concentration range of 0.1-100 μg/ml diluted with sterile distilled water from a 1mg/ml stock solution of the pure compounds in DMSO (0.1 ml), with the highest content of DMSO in any well at 2.5%. The cells were incubated at 30^{0} for 24 hours (for Candida spp. and Trichosporon cutaneum and 48 hours (for Cryptococcus neoformans strains and Turolopsis glabrata) and the MIC reading recorded at 24 hours and 48 hours respectively using a colony reader compared to control cultures incubated on the same plate at the same conditions above, and in duplicate sets.

The results shown in Table 15 compare the tested compounds against two established clinical agents, Isoconazole and Amphotericin B which are commonly used for the control of human fungal infections caused by Candida albicans and Cryptococcus neoformans. The strains used in the tests are identified in the Table as follows:

Cryptococcus neoformans	T39/t
Cryptococcus neoformans	T31/a
Cryptococcus neoformans	T65/j
Cryptococcus neoformans	CW/4
Cryptococcus neoformans	CW/1
Candida albicans	sh/27
Candida parapsilosis	jh/69
Candida tropicalis	ah/54
Turolopsis glabrata	MMa/13
Trichosporon cutaneum	Kat/37

These pathogenic fungi are commonly associated with superficial and systemic fungal infections. The above mentioned strains are officially recognised strains which, for the purposes of these tests, were obtained from the Department of Pharmacology and Microbiology, University of Kuwait Faculty of Medicine, Sasat, Hawalli, 32074 Kuwait.

Table 15:

	MIC (μg/ml)									
	C. Neoformans Strains					Candida				
Compound	T39/t	T31/a	T65/j	CW/4	CW/1	sh/27	jh/69	ah/54	MMa/13	Kat/37
Isoconazole	12.5	12.5	12.5	100.0	125.0	12.5	25.0	6.0	12.5	1.5
Amphotericin B	6.0	1.5	3.0	-	-	3.0	50.0	25.0	3.0	25.0
Example 250	12.5	25.0	25.0	25.0	25.0	25.0	25.0	25.0	12.5	12.5
Example 251	12.5	25.0	25.0	12.5	25.0	25.0	25.0	25.0	12.5	25.0
Example 252	12.5	12.5	12.5	12.5	25.0	12.5	25.0	25.0	12.5	25.0
Example 253	25.0	25.0	50.0	50.0	25.0	50.0	50.0	50.0	50.0	12.5
Example 254	12.5	25.0	25.0	25.0	25.0	25.0	25.0	50.0	25.0	25.0
Example 255	6.0	12.5	6.0	6.0	6.0	12.5	12.5	25.0	6.0	6.0
Example 238	6.0	6.0	12.5	6.0	6.0	12.5	12.5	25.0	12.5	12.5
Example 239	12.5	12.5	12.5	25.0	25.0	12.5	12.5	25.0	12.5	12.5

Table 15: Contd/..

	C. Neoformans Strains					Candida				
Example	T39/t	T31/a	T65/j	CW/4	CW/1	sh/27	jh/69	ah/54	MMa/13	Kat/37
240	25.0	12.5	25.0	12.5	12.5	50.0	50.0	50.0	25.0	12.5
241	12.5	6.0	6.0	3.0	6.0	25.0	50.0	25.0	12.5	12.5
256	50.0	50.0	100.0	100.0	100.0	125.0	100.0	100.0	50.0	25.0
242	100.0	100.0	50.0	50.0	50.0	25.0	25.0	12.5	3.0	6.0
243	12.5	12.5	12.5	25.0	25.0	25.0	50.0	50.0	50.0	12.5
244	12.5	12.5	12.5	25.0	12.5	25.0	25.0	25.0	12.5	12.5
245	6.0	3.0	6.0	1.5	3.0	6.0	25.0	25.0	3.0	6.0
257	6.0	6.0	12.5	12.5	6.0	25.0	50.0	50.0	25.0	6.0
258	25.0	25.0	25.0	12.5	25.0	25.0	50.0	25.0	25.0	25.0
249	6.0	6.0	6.0	3.0	6.0	12.5	6.0	6.0	6.0	6.0

Table 15: Contd/..

Example	C. Neoformans Strains					Candida				
	T39/t	T31/a	T65/j	CW/4	CW/1	sh/27	jh/69	ah/54	MMa/13	Kat/37
248	3.0	6.0	6.0	3.0	6.0	12.5	25.0	25.0	6.0	3.0
261	1.5	6.0	3.0	6.0	6.0	12.5	12.5	12.5	6.0	1.5
262	3.0	6.0	6.0	3.0	6.0	12.5	25.0	25.0	3.0	6.0
263	6.0	6.0	6.0	3.0	6.0	12.5	25.0	25.0	6.0	6.0
259	6.0	6.0	6.0	12.5	6.0	12.5	12.5	12.5	6.0	3.0
246	25.0	50.0	50.0	50.0	25.0	50.0	50.0	50.0	25.0	50.0
264	12.5	25.0	25.0	12.5	12.5	125.0	175.0	150.0	100.0	12.5
265	6.0	6.0	6.0	6.0	3.0	12.5	12.5	12.5	6.0	6.0
266	3.0	6.0	6.0	6.0	6.0	6.0	12.5	12.5	12.5	12.5
267	25.0	12.5	12.5	12.5	25.0	25.0	50.0	25.0	12.5	12.5

Antibiotic Activity

Bacteria used in screen:

Klebsiella aerogenes
Escherichai coli (18) (Gram negative)
Pseudomonas aeruginosa (Kirke) (Gram negative)
Klebsiella pneumonia (15883) (Gram negative, -lactamase producing strain)
Bacillus subtilis (Oxford) (Gram positive).
Staphylococcus aureus (Oxford) (Gram positive).

The bacteria chosen are those usually employed in routine screening of compounds for potential antibacterial activity, being representative of Gram positive, Gram negative and beta-lactamase producing organisms. (Reference: British Pharmacopoeia 1980, Vol. 11, Section 19122, Appendix XIV, Biological Assay of Antibiotics). Control antibiotics were used in the form of Oxoid "multidisk" incorporating chloramphenicol, erythromycin, penicillin G, streptomycin and tetracycline.

Antibiotic Testing Procedure

The following materials were employed:

Bacteria

Escherichia coli (18)
Pseudomonas aeruginosa (Kirke)
Klebsiella pneumonia (15883)
Bacillus subtilis (Oxford)
Staphylococcus aureus (Oxford)

Medium (pH = 7.4)

Oxoid nutrient agar (CH_3) containing:

Lab-Lemco powder (oxoid L29)	1g/litre
Yeast extract (oxoid L20)	2g/litre
Peptone (oxoid L39)	5g/litre
NaCl	5g/litre

Oxoid nutrient broth

NaCl:	5g
Bacto Tryptone:	10g
Yeast extract:	5g
Deionized water to	1 litre

Assay

Day 1

Using a sterile loop petri dishes were streaked with the chosen bacteria and were incubated overnight at 37°C.

Day 2

Overnight cultures were prepared by innoculating single colonies (obtained from the previously prepared petri dishes) in 2-3 ml of broth using

a sterile loop and shaking overnight in a water bath at 37°C.

Method

Each overnight culture (10µl) was added and mixed to the oxoid nutrient broth (10 ml). The resultant mixture (3 mls) was poured and swirled onto a petri dish containing the set antibiotic medium. The excess liquid was removed with a sterile dropper and the petri dish was left in a laminar air flow hood for 20 mins to dry.

A solution or suspension of each compound to be tested was prepared at concentrations of 10, 5, 2.5 and 1.2 mg/ml in water. The resultant solution (25µl) was dispersed onto Whatman's No. 1 filter paper discs of diameter 5mm and allowed to air dry. The treated discs were placed on each agar plate and the plates were incubated at 37° for 24 hr. Zones of inhibition were then measured and recorded.

All tests were carried out in duplicate.

Estimate of minimum inhibitance concentration (MIC) value.

By decreasing the concentration of active sample placed on the filter paper disc a concentration at which no inhibitance occurs was observed and the MIC value was then recorded.

For clinically useful antibiotics e.g Ampicillin, Cephalexin, Tetracycline and Erythromycin MIC values are ≤ 100 µg/ml = 0.1 mg/ml.

Table 16: Anti-bacterial Activity

Compound Calixarene	E. coli	S. aureus	Klebsiella	B. subtilus	Ps aeruginosa	Concentration
Example 268	-	-	XXXX	XXX	XXXX	10mg/ml
	-	-	X	X	XXX	5mg/ml
	-	-	-	-	X	1mg/ml
Example 269	-	-	-	XXXX	X	10mg/ml
	-	-	-	XX	-	5mg/ml
	-	-	-	X	-	1mg/ml
Example 270	-	-	XXXX	-	-	10mg/ml
	-	-	XXX	-	-	5mg/ml
	-	-	X	-	-	1mg/ml
Example 271	X	XXX	XXX	X	XXX	10mg/ml
	-	XX	XX	X	XX	5mg/ml
	-	XX	XX	-	XX	1mg/ml
	-	X	XX	-	-	0.5mg/ml

Table 16: Anti-bacterial Activity

Compound Calixarene	E. coli	S. aureus	Klebsiella	B. subtilus	Ps aeruginosa	Concentration
Example 242	-	-	-	XXX	XXX	2mg/ml
Example 249	XX	XX	-	-	-	2.5mg/ml
Example 248	-	-	-	-	-	10mg/ml
Example 260	-	-	-	-	-	10mg/ml

Table 16: Anti-bacterial Activity

Compound Calixarene	E. coli	S. aureus	Klebsiella	B. subtilus	Ps aeruginosa	Concentration
Example 272	-	XX	XXXX	-	-	10mg/ml
	-	X	XXX	-	-	5mg/ml
	-	-	XXX	-	-	1mg/ml
	-	-	XX	-	-	0.5mg/ml
Example 273	XXXX	XXXX	-	-	-	10mg/ml
	XXXX	XXXX	-	-	-	5mg/ml
	XX	XX	-	-	-	1mg/ml
	-	X	-	-	-	0.5mg/ml
Example 274	-	XXXX	X	XXXX	-	10mg/ml
	-	XXXX	-	XXX	-	5mg/ml
	-	XXX	-	XX	-	1mg/ml
	-	X	-	X	-	0.1mg/ml

Table 16: Anti-bacterial activity

Compound Calixarene	E. coli	S. aureus	Klebsiella	B. subtilus	Ps aeruginosa	Concentration
	-	XXXX	-	XXXX	-	10mg/ml
Example 275	-	XXXX	-	XXXX	-	5mg/ml
	-	X	-	XX	-	1mg/ml
	-	-	-	X	-	0.1mg/ml
	-	XX	-	XXX	-	10mg/ml
Example 74	-	X	-	X	-	5mg/ml
	-	-	-	-	-	1mg/ml
	-	-	-	-	-	0.1mg/ml

Key

- : No zone of inhibition observed around the 5 mm disc.
X : Zone of inhibition, total diameter (disc and zone) = 6 mm.
XX: Zone of inhibition, total diameter (disc and zone) = 7 mm.
XXX: Zone of inhibition, total diameter (disc and zone) = 10 mm.
XXXX Zone of inhibition, total diameter (disc and zone) = 14 mm.

Anti-Cancer Activity

Anti-cancer activity of compounds was assessed using the screening methodology of Boyd (18). Figures 1 to 7 of the drawings show the IC_{50} mean graphs for the compounds of Examples 32, 248, 261, 272, 276, 277 and 282 respectively. The IC_{50} is a measure of the relative cell line sensitivities to the compound tested and is determined by comparing the relative compound concentrations required to produce the same level of response in each cell line.

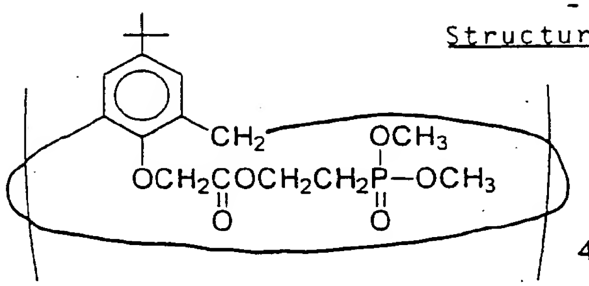
Following the methodology of Clynes (19) compound 276 appeared not to be effluxed by mdr-1 (multidrug resistance)-associated 170 p glycoprotein as it was tested against human lung squamous cell line DLKP and 300 fold adriamycin-resistant variant DLKP-A. IC_{50} (inhibitory concentration giving reduction in growth 50%) were found to be 1.47 µm and 1.66 µm respectively for DLKP and DLKP-A. Little difference in toxicity for DLKP compared to DLKP-A indicates this compound 276 not transported by the p-glycoprotein.

Compound of Example 276

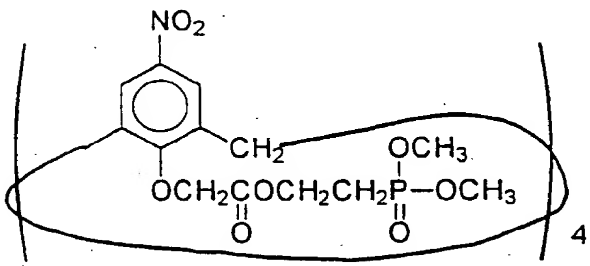
	DLKP	DLKP-A
NC1-2 Toxicity range	R1 0---2.5µg/ml IC50 1.75µg/ml	R1 0---3µg/ml IC50 1.95µg/ml
	R2 0---2.9µg/ml IC50 1.85µg/ml	R2 0----3.25µg/ml IC50 2.1µg/ml
	R3 0---2.4µg/ml IC50 1.53µg/ml	R3 0----3.25µg/ml IC50 1.75µg/ml

Observation: No difference in toxicity was observed for DLKP compared to DLKP-A. This would indicate that this particular agent is not transported by the P-glycoprotein as no differential toxicity is seen. The results were fairly consistent with the error within the acceptable ranges.

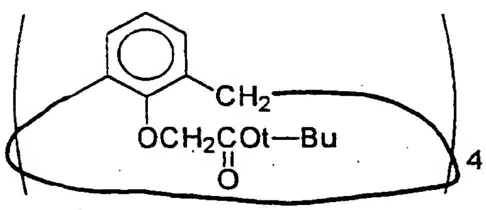
Structural Formulae



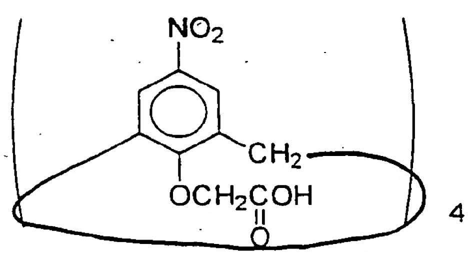
Compound 12



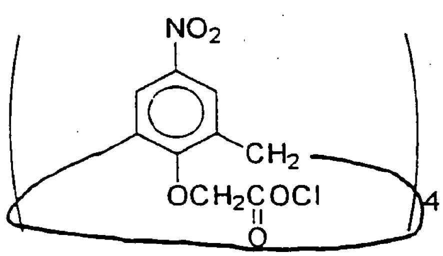
Compound 13



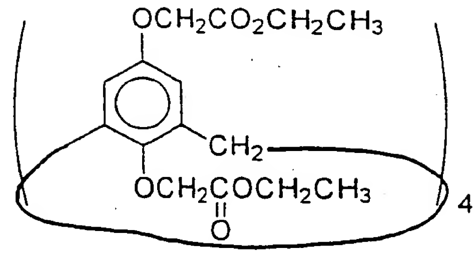
Compound 13a



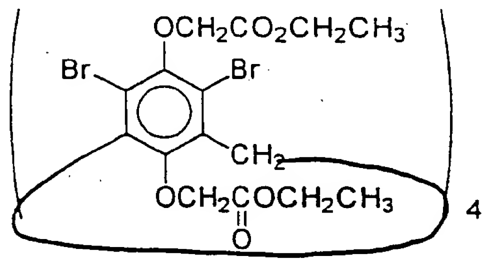
Compound 13b



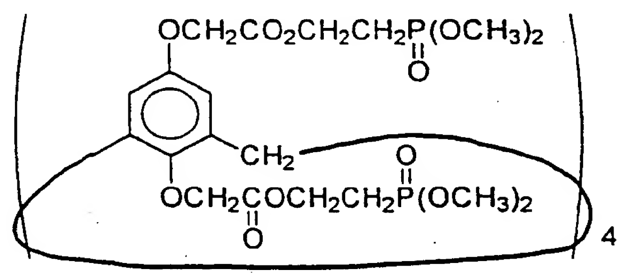
Compound 13c



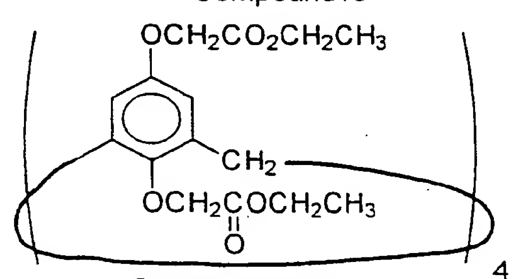
Compound 14



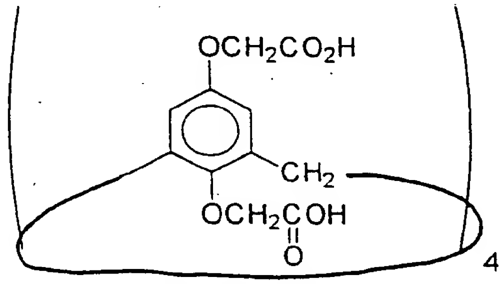
Compound15



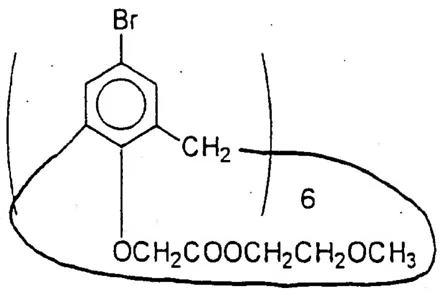
Compound16



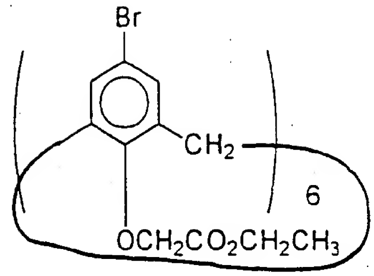
Compound 16a



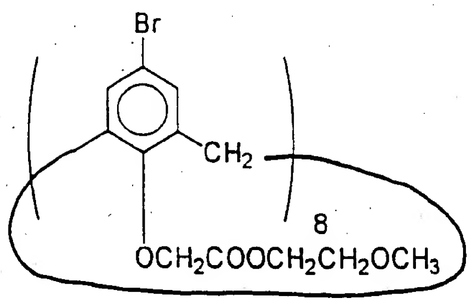
Compound 16b



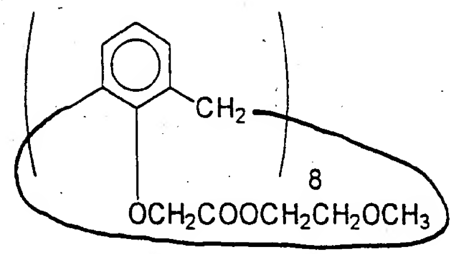
Compound 18



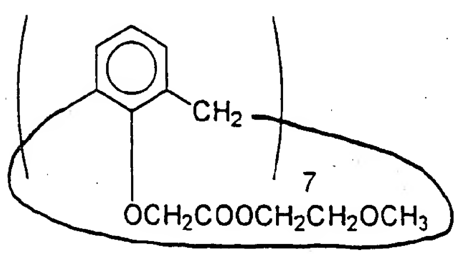
Compound18a



Compound 19



Compound 20



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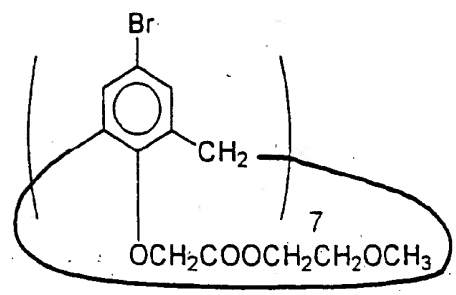
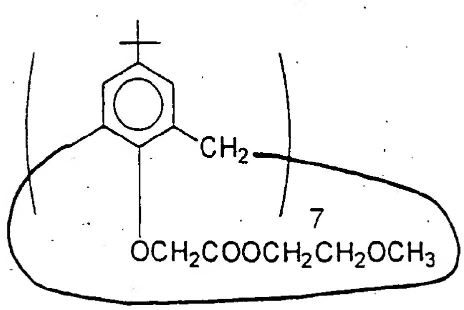
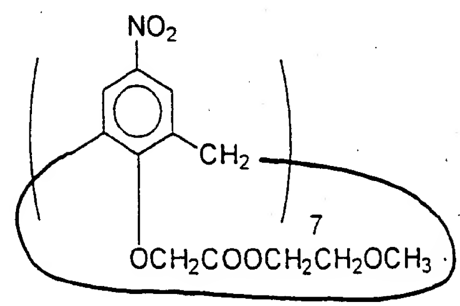


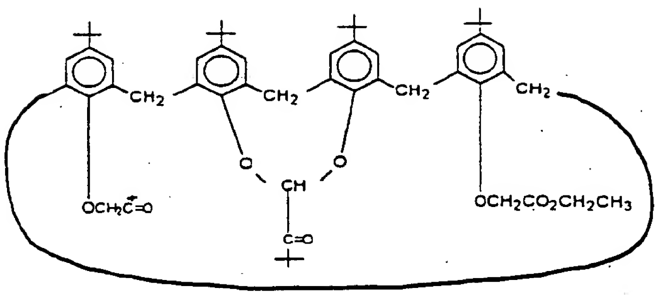
Compound 22



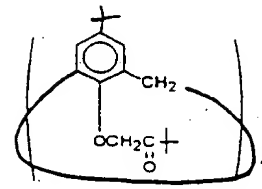
Compound 23



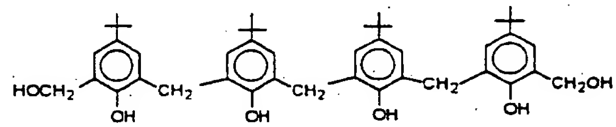
Compound 24



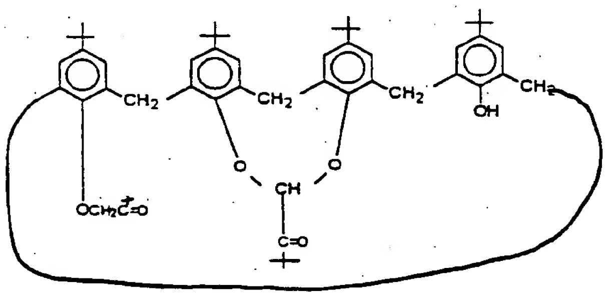
Compound 17



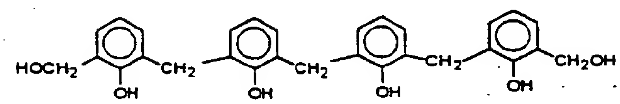
Compound 17a



Compound 96



Compound 17b



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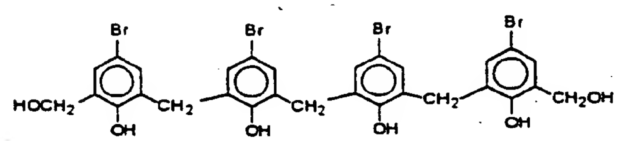

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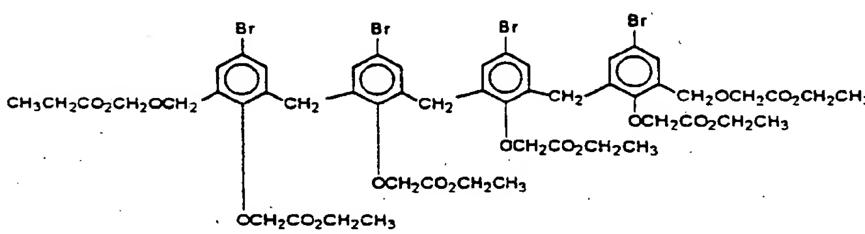

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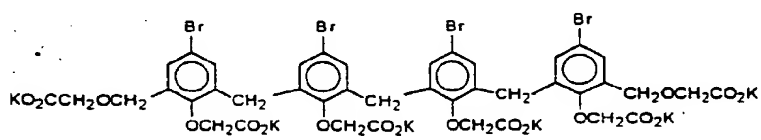

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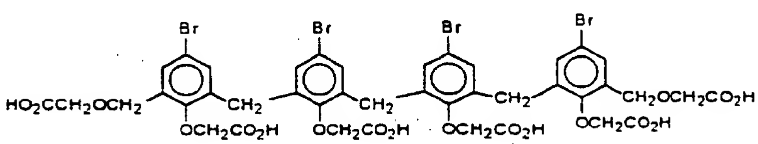

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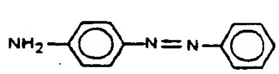
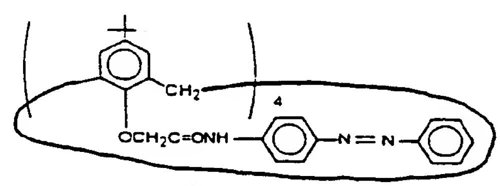
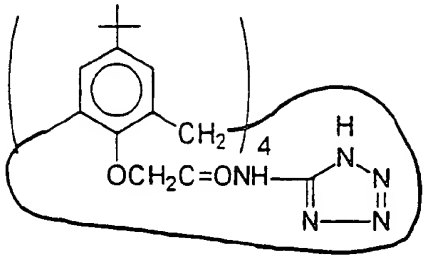


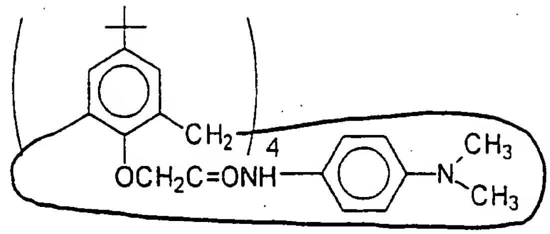
Compound 102a



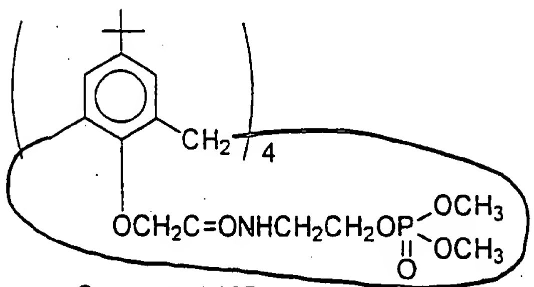
Compound 102



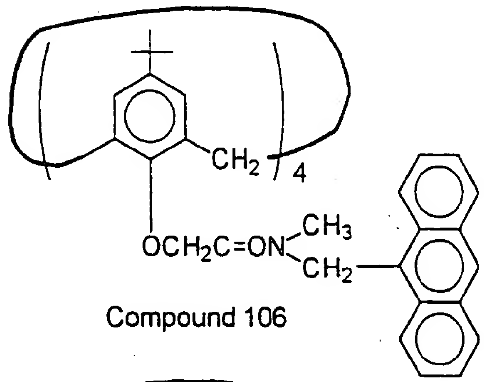
Compound 103



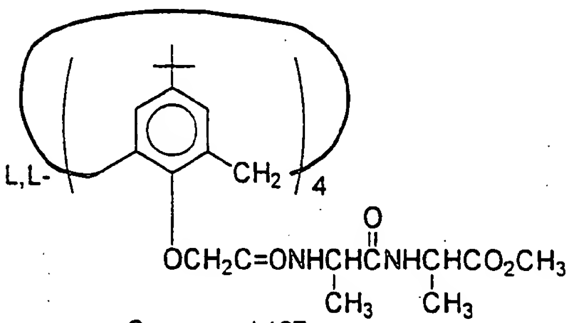
Compound 104



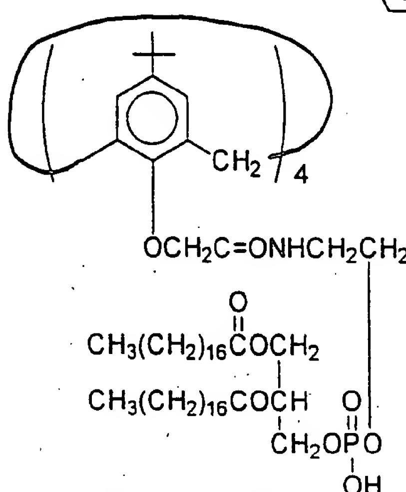
Compound 105



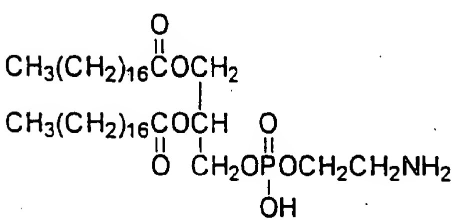
Compound 106



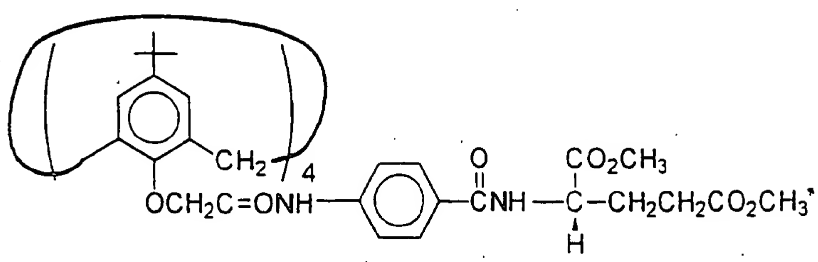
Compound 107



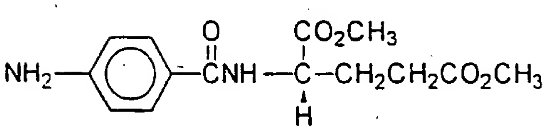
Compound 108



Compound 108a



Compound 109



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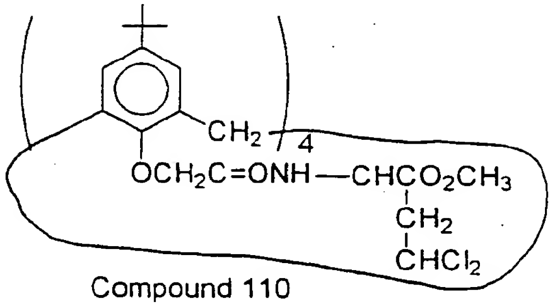

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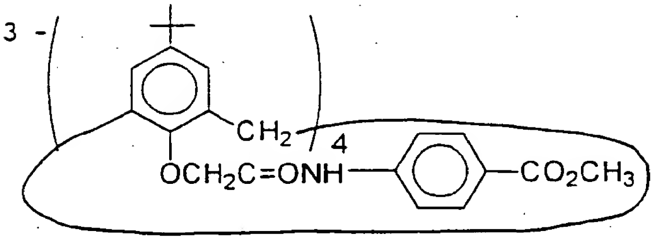

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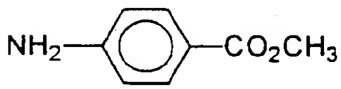

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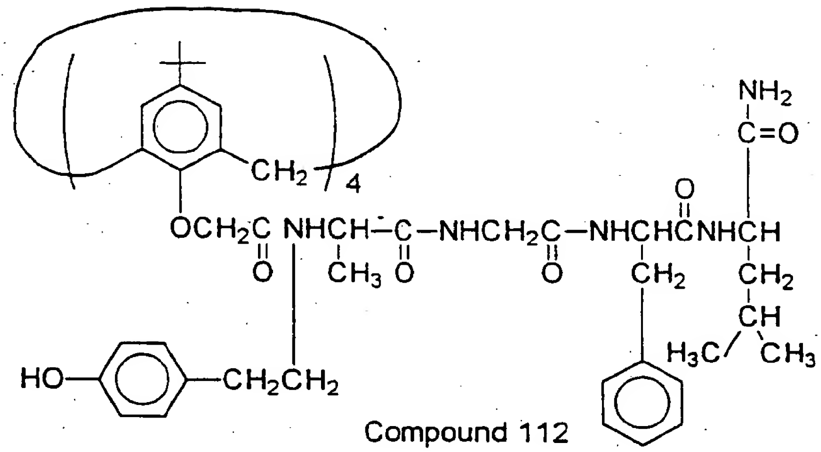

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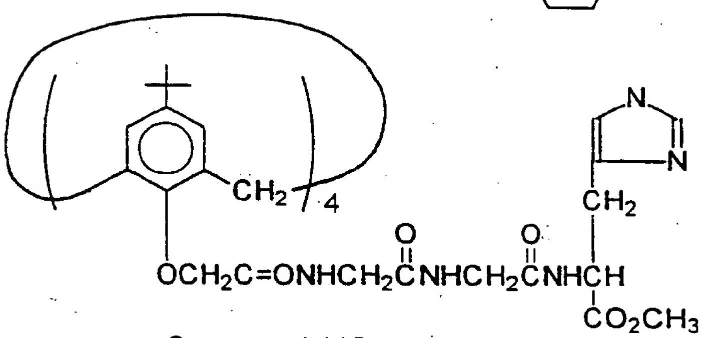

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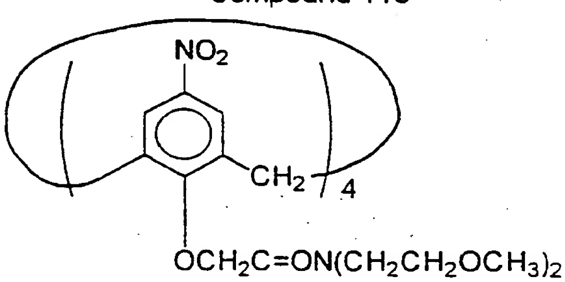

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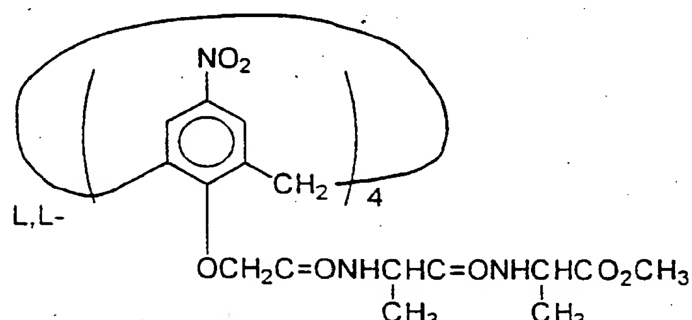

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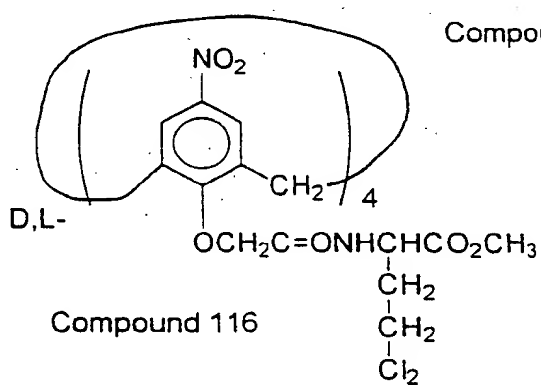

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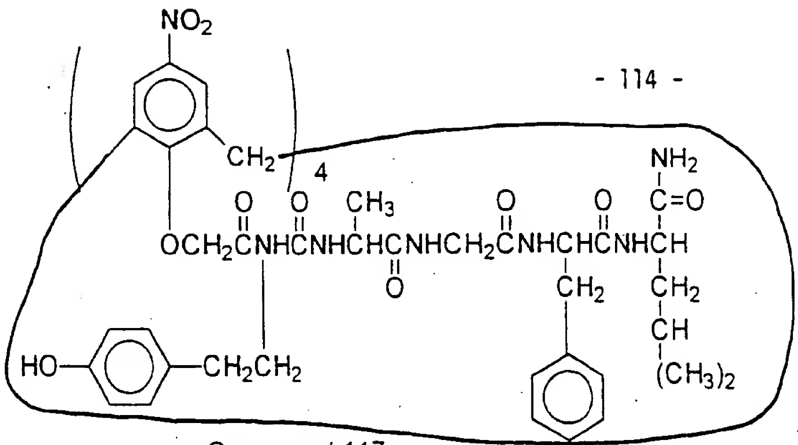

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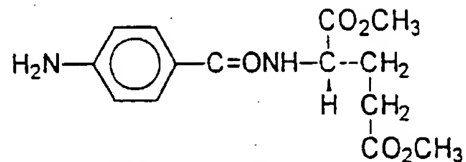

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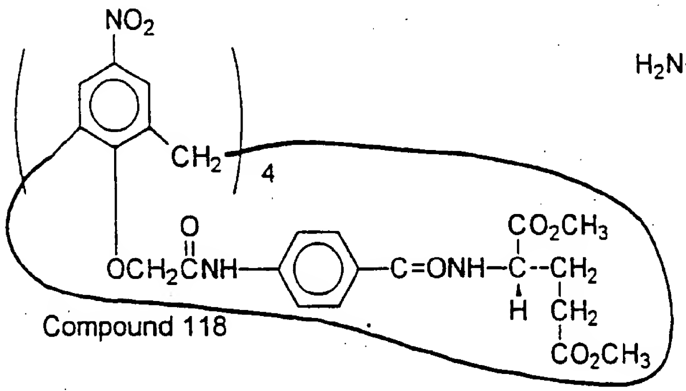

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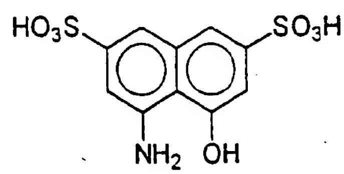

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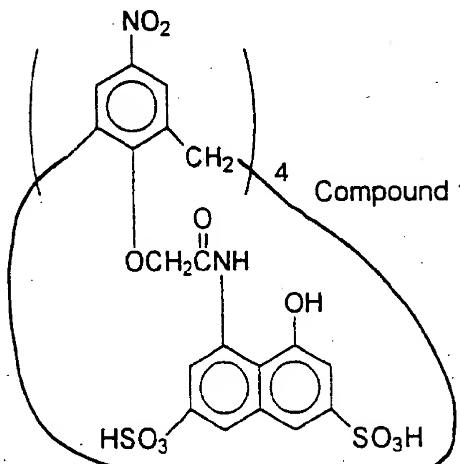

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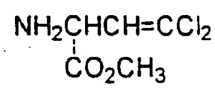

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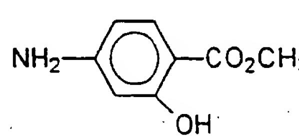

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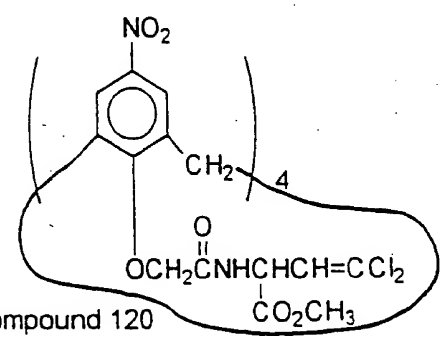

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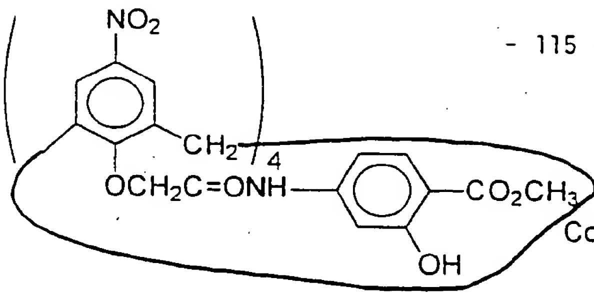

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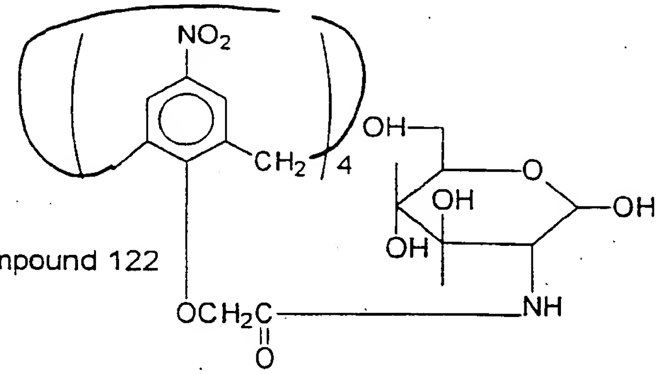

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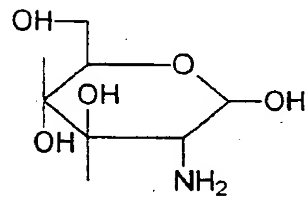

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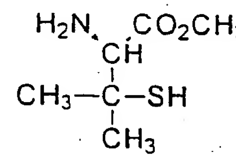

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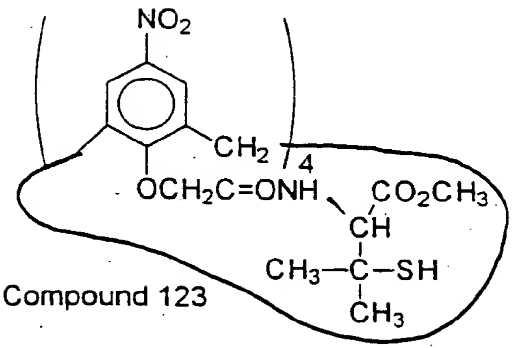

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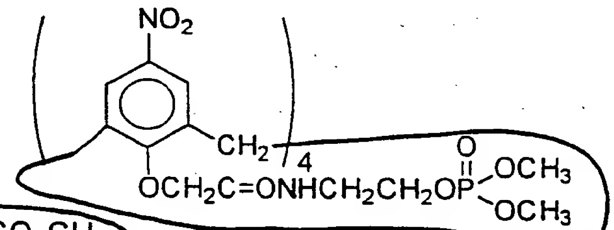

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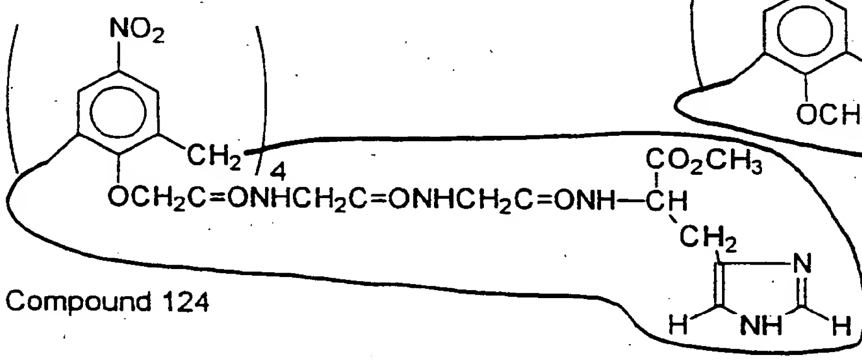

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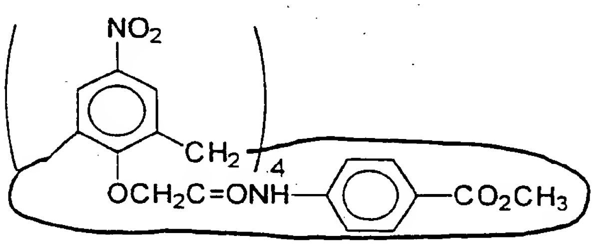

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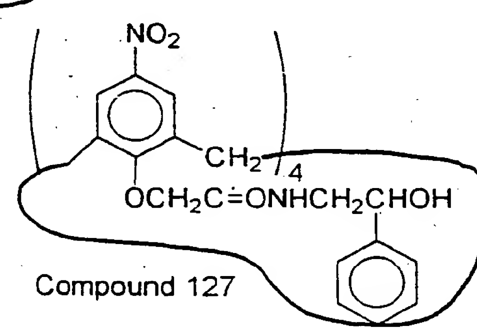

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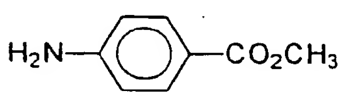

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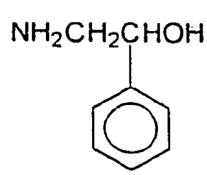

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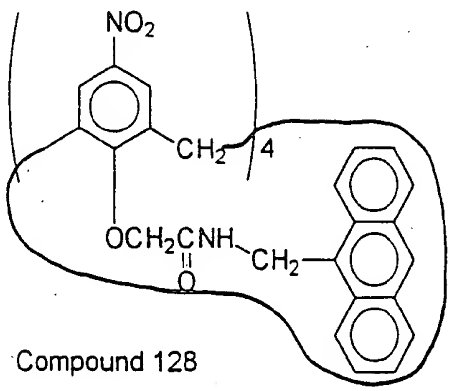

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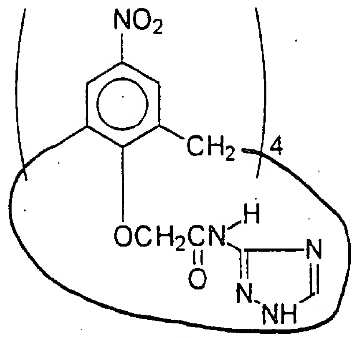

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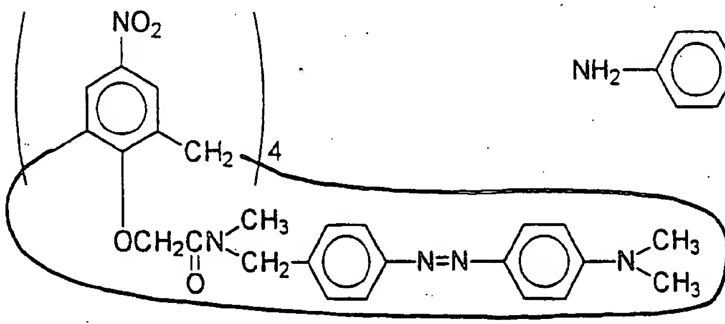

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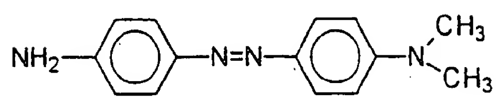

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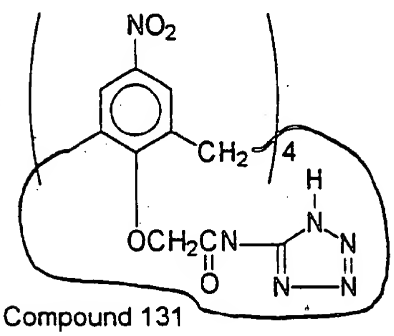

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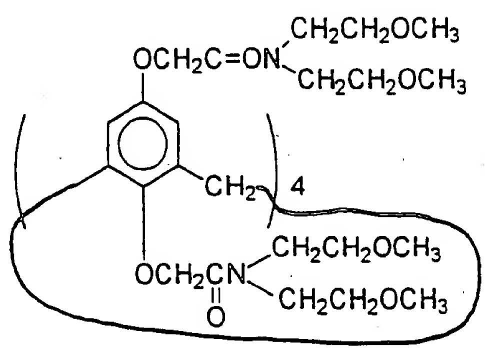

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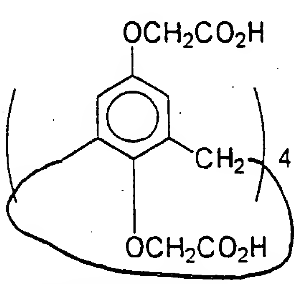

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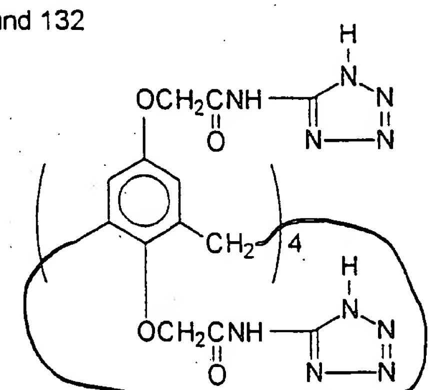

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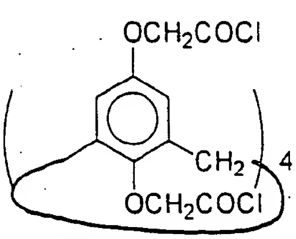

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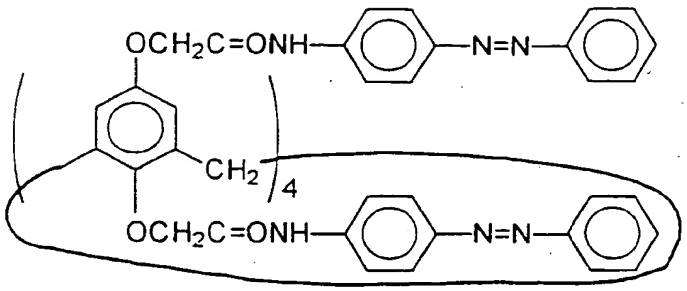

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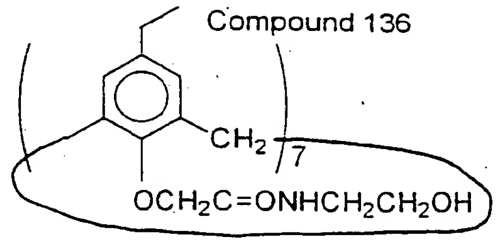

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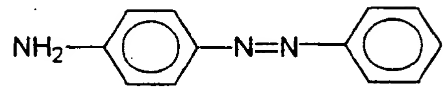

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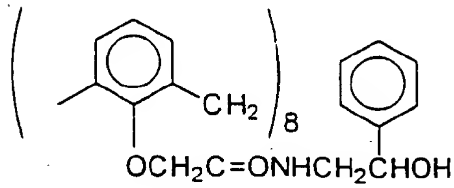

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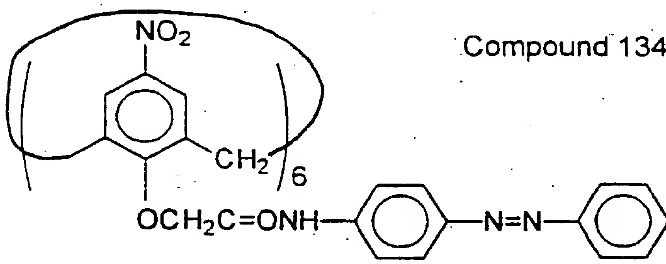

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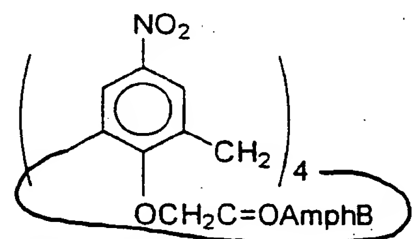

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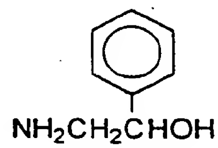

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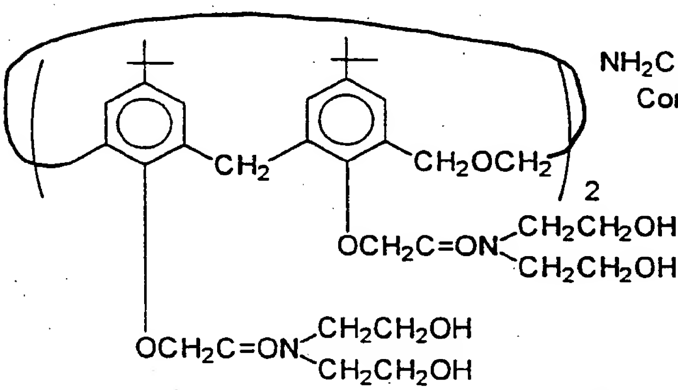

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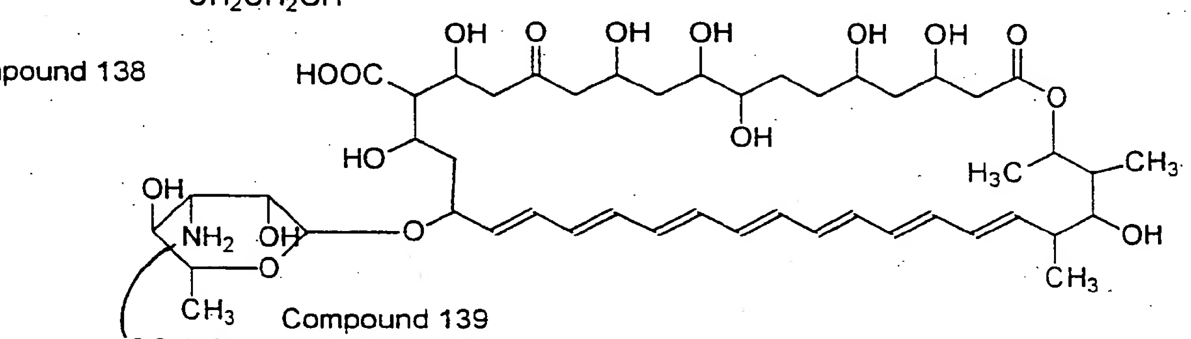

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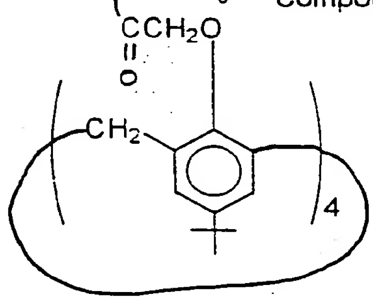

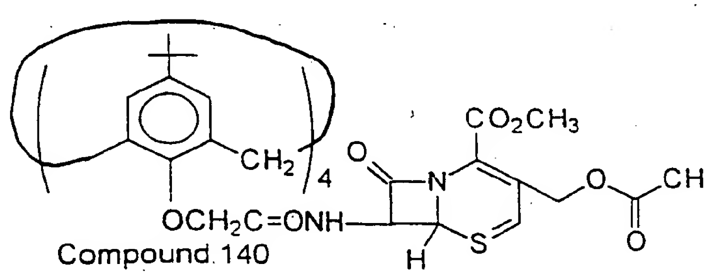

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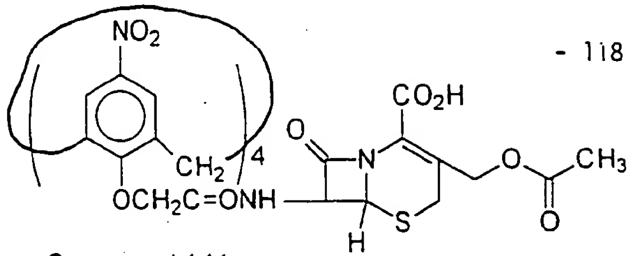

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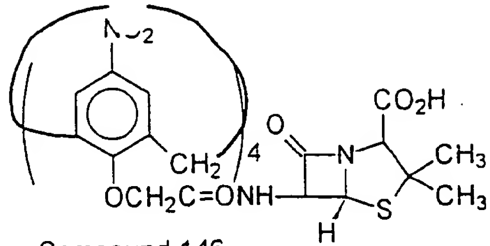

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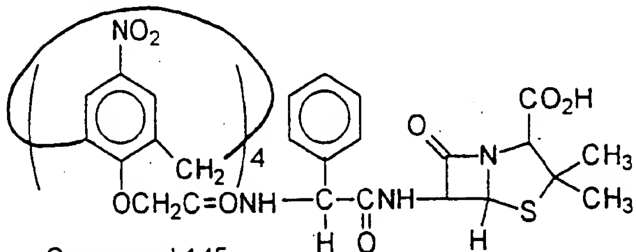

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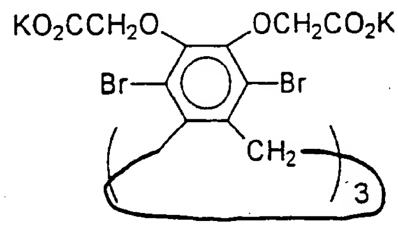

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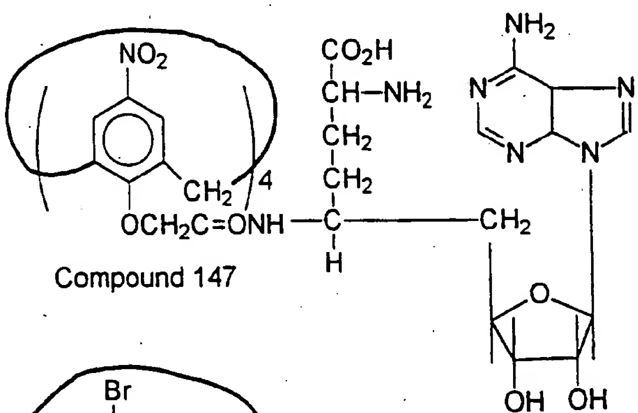

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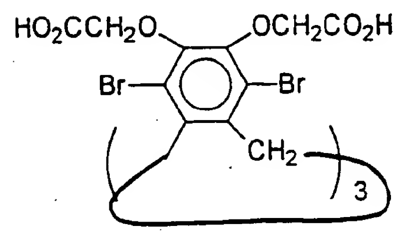

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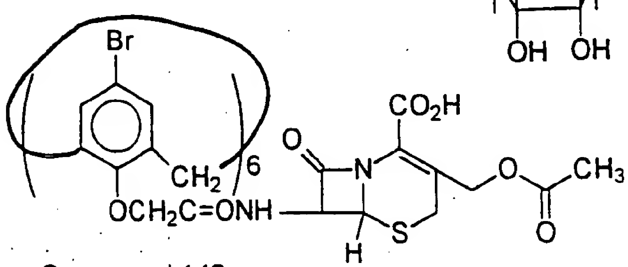

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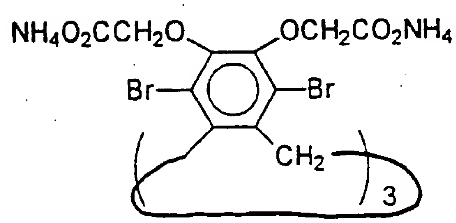

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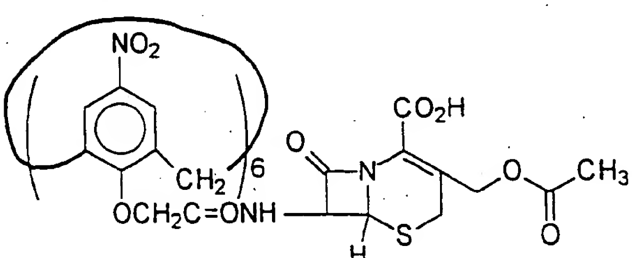

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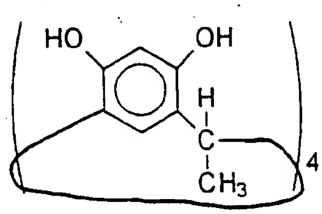

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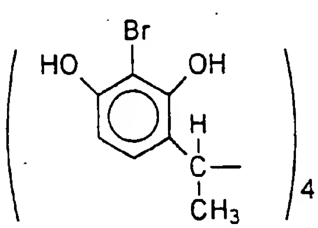

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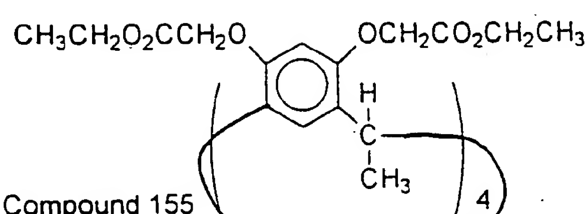

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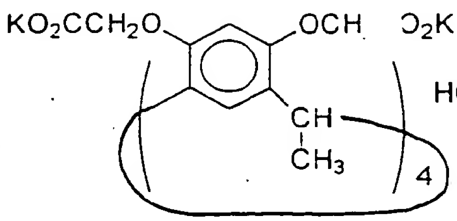

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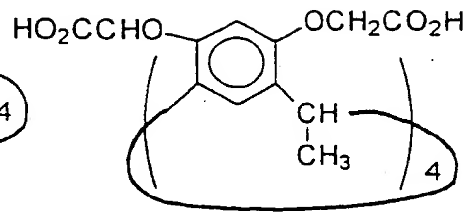

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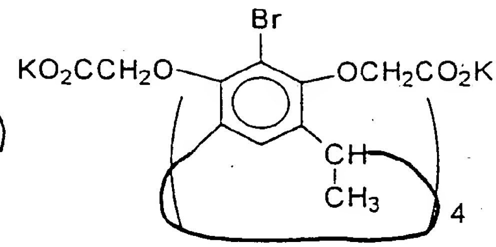

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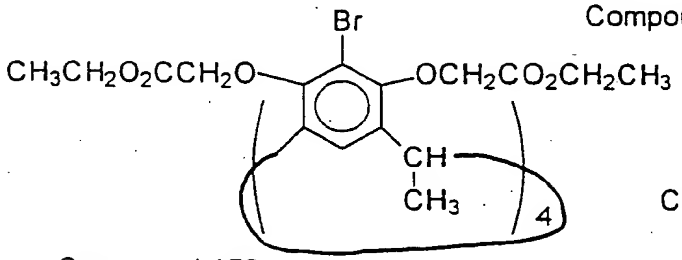

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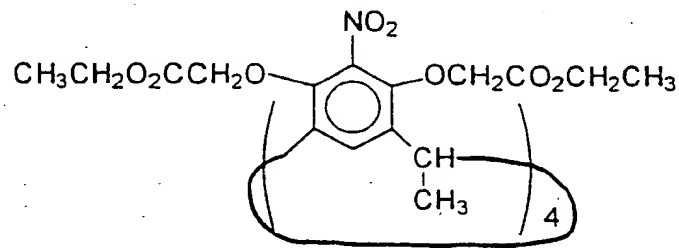

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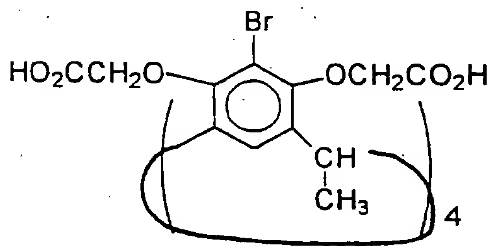

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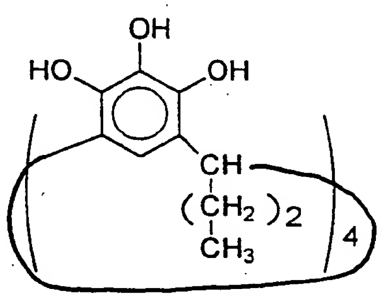

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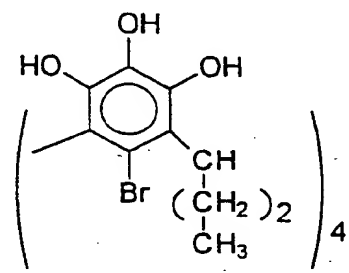

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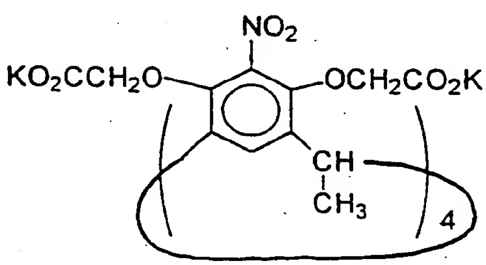

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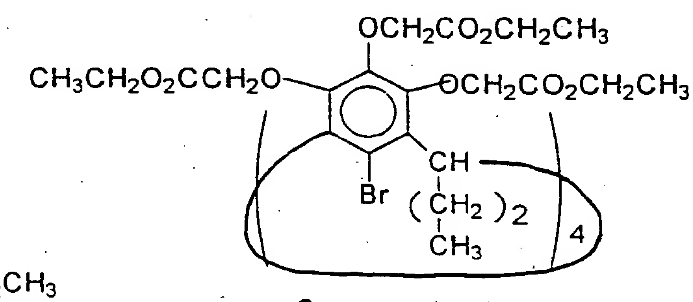

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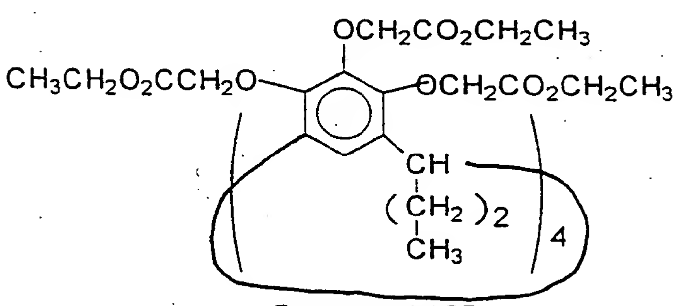

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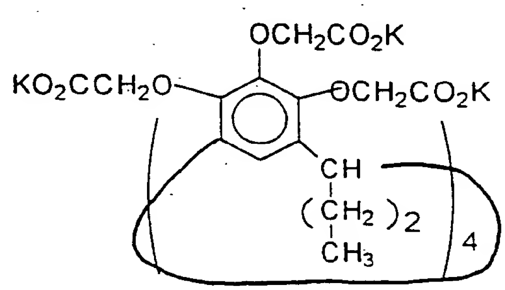

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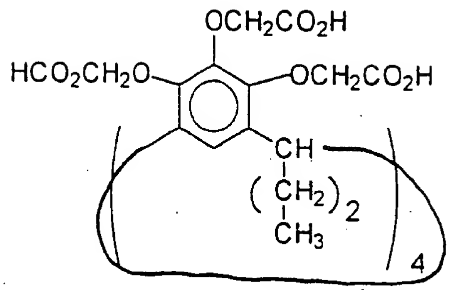

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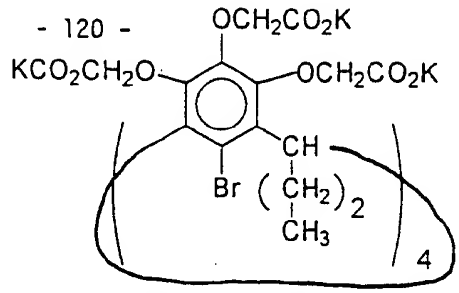

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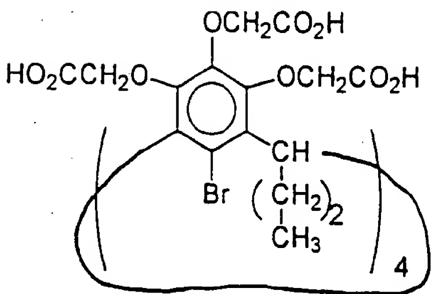
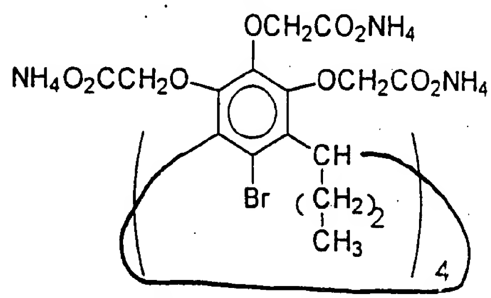


Compound 170



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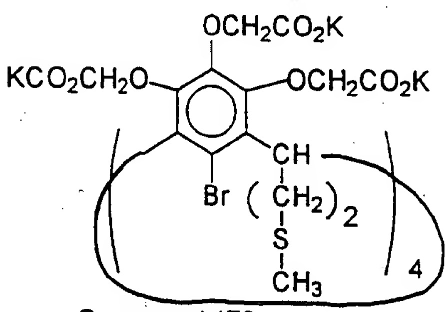

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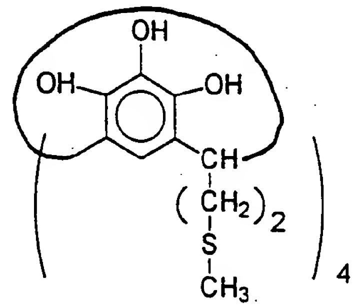

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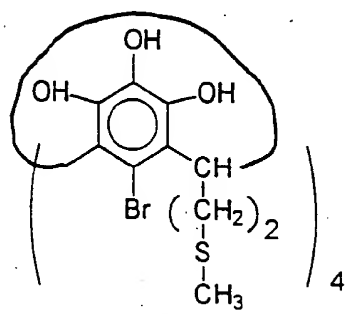

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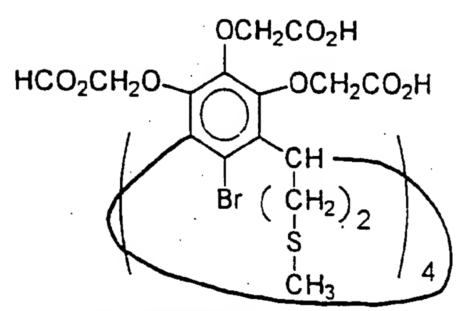

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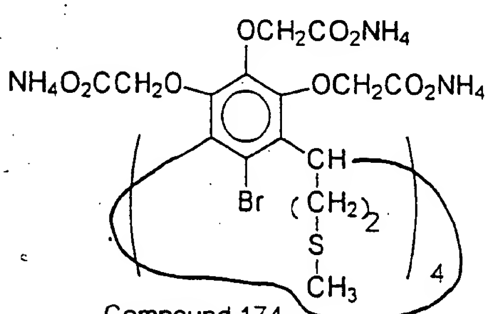

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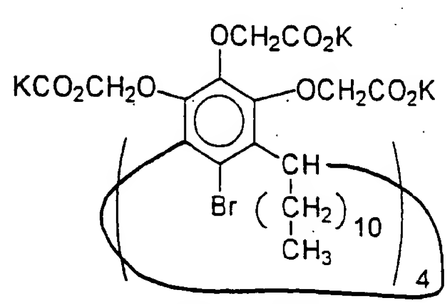

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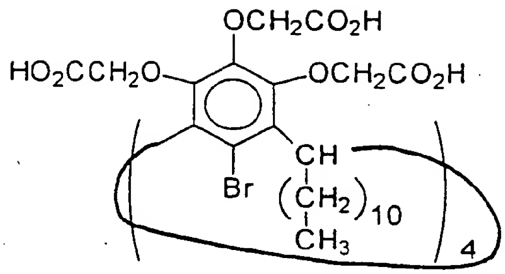

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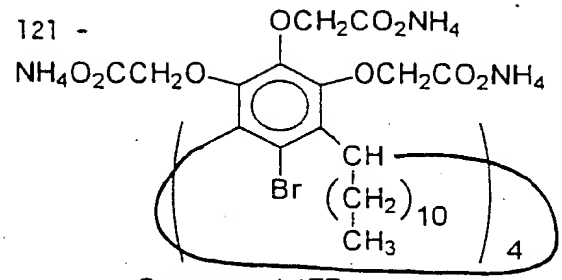

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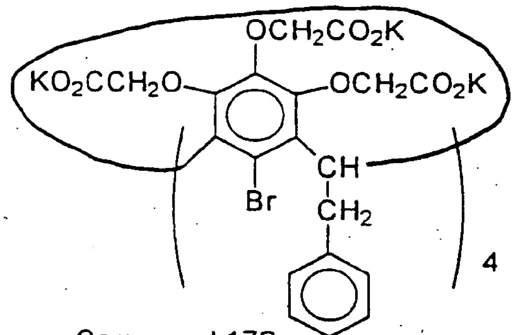

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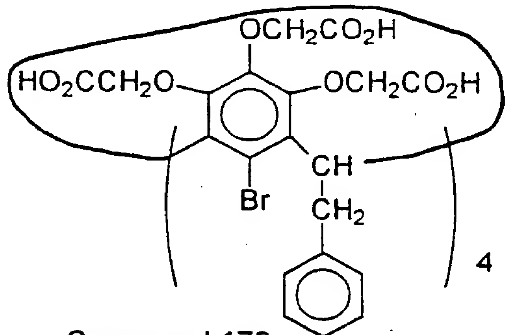

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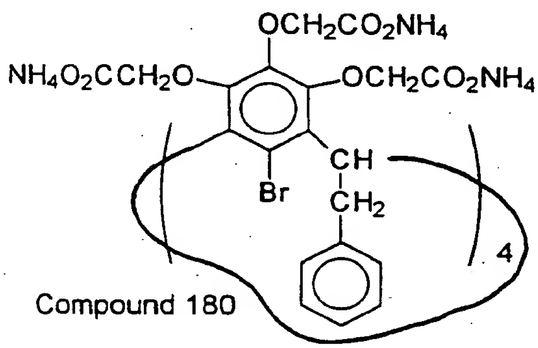

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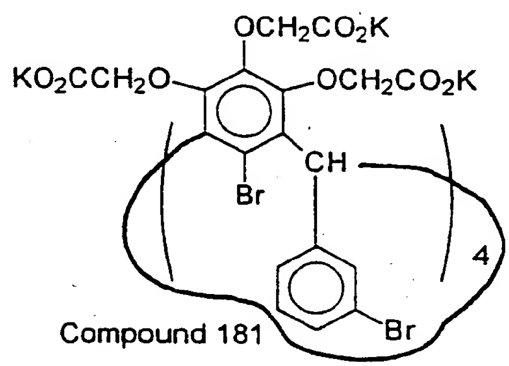

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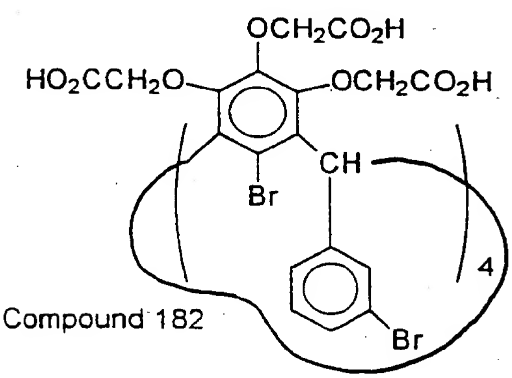

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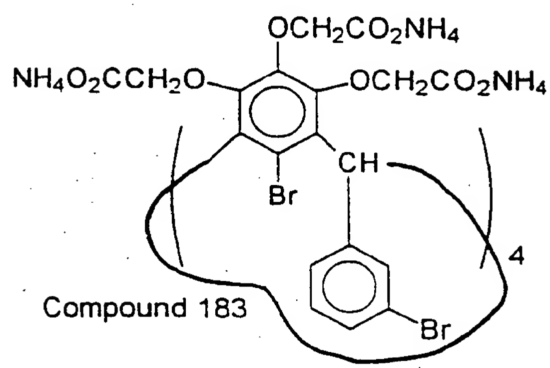

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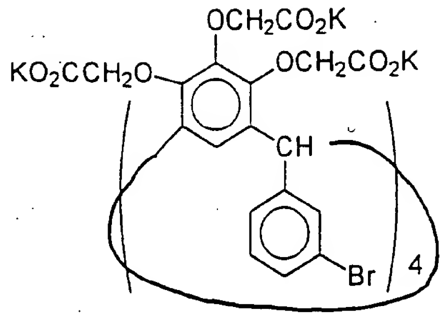

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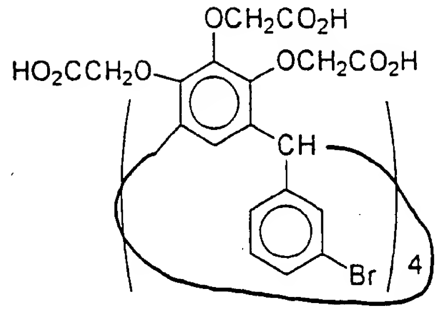

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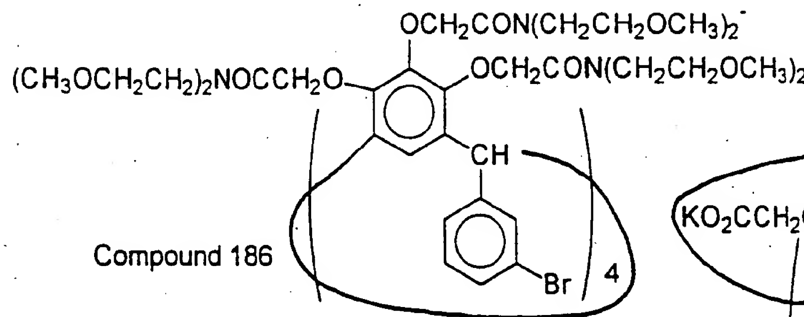

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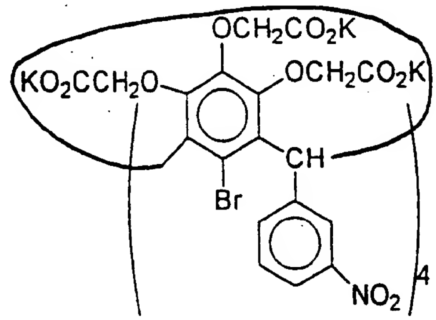

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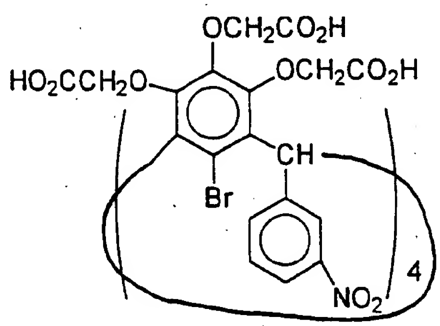

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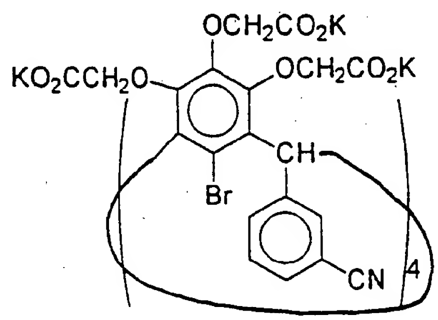

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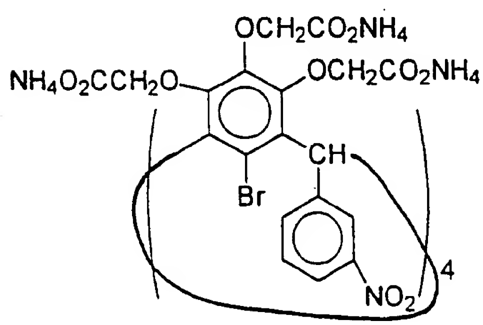

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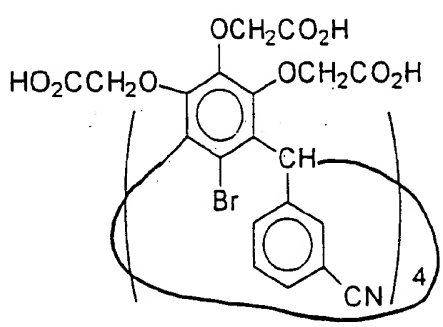

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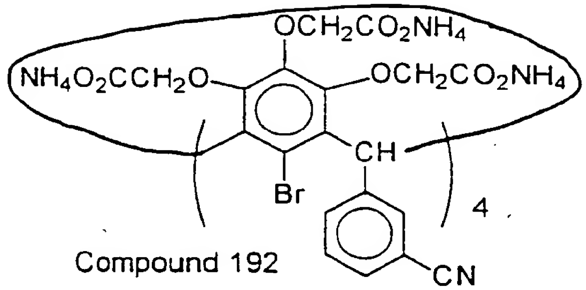

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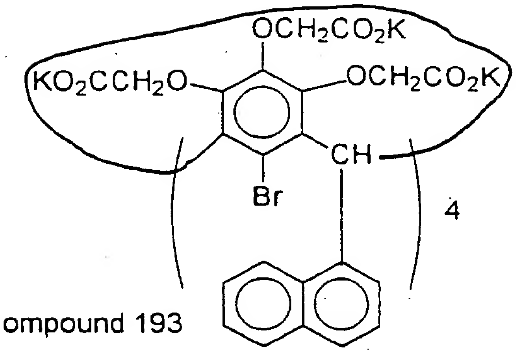

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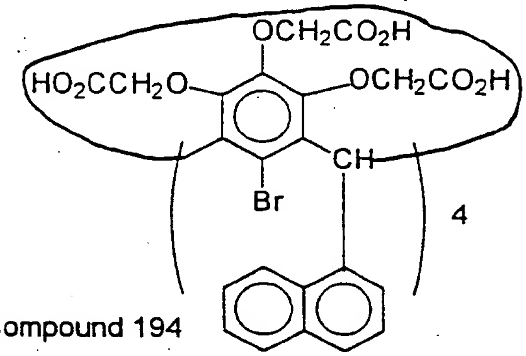

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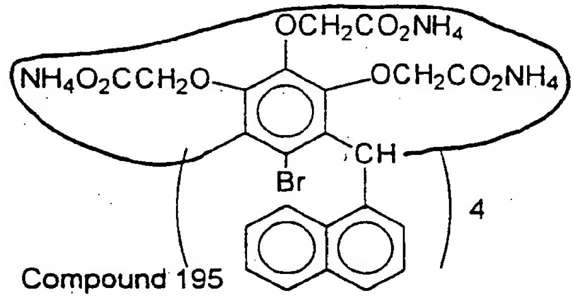

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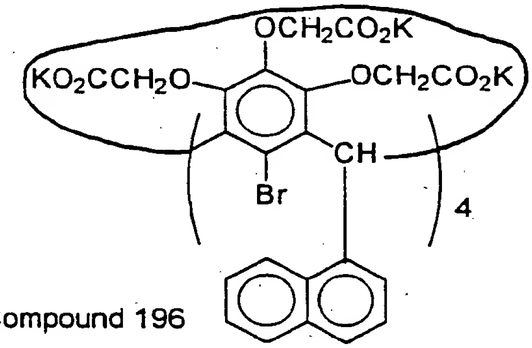

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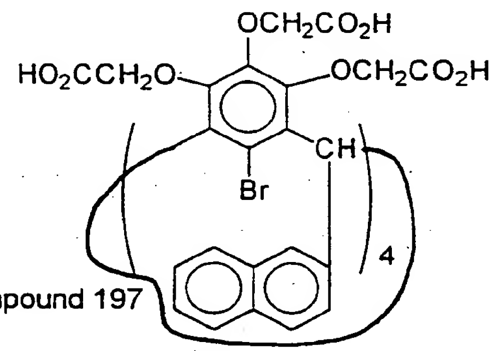

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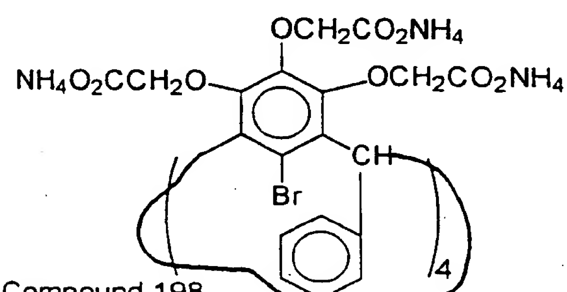

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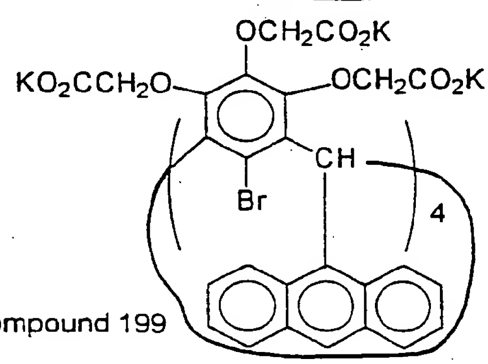

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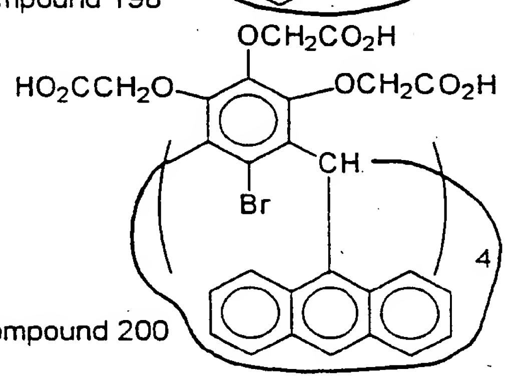

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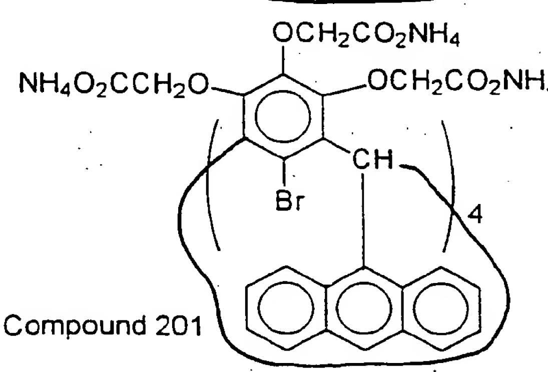

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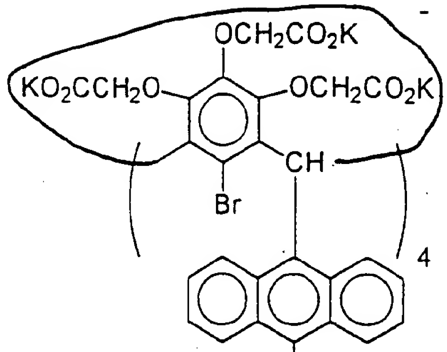

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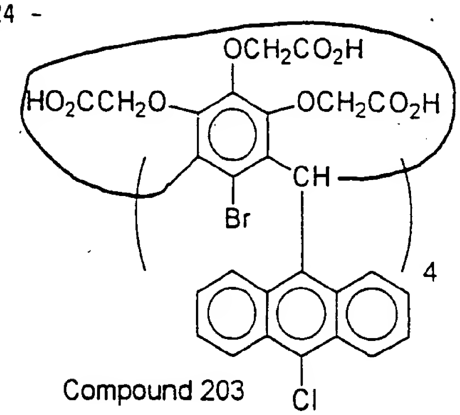

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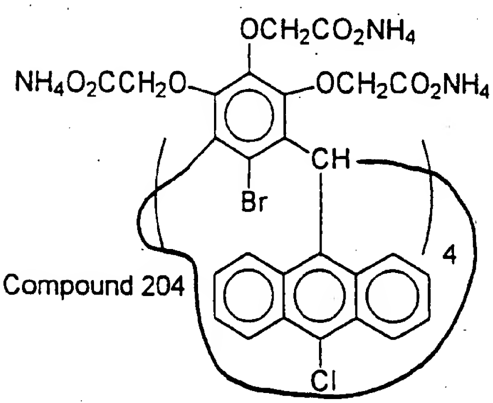

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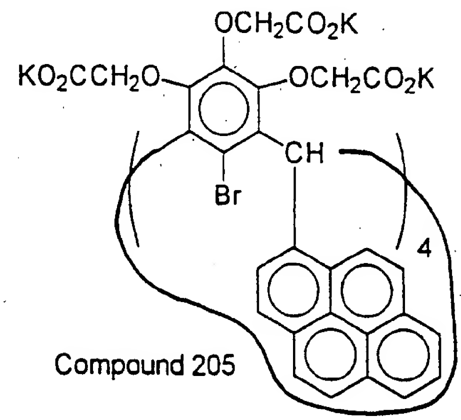

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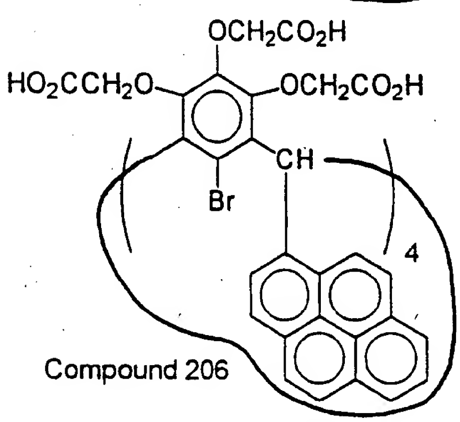

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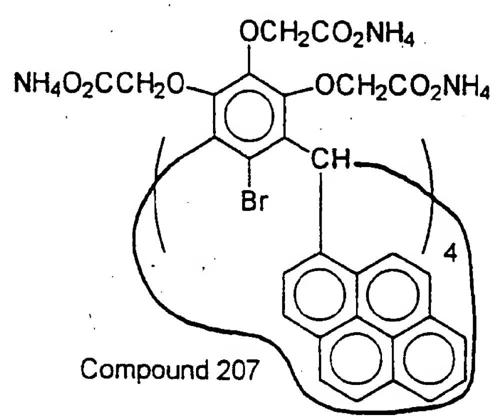

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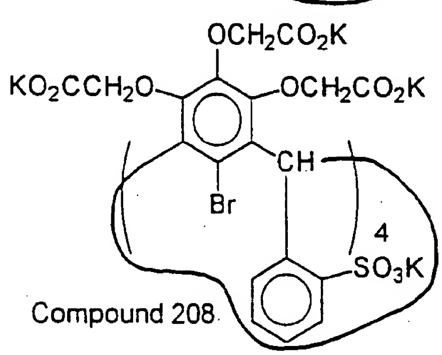

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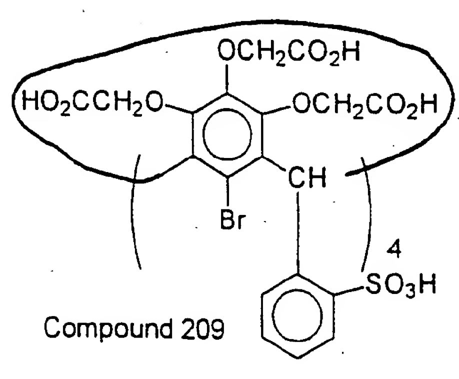

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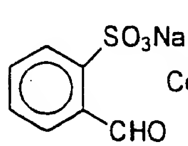

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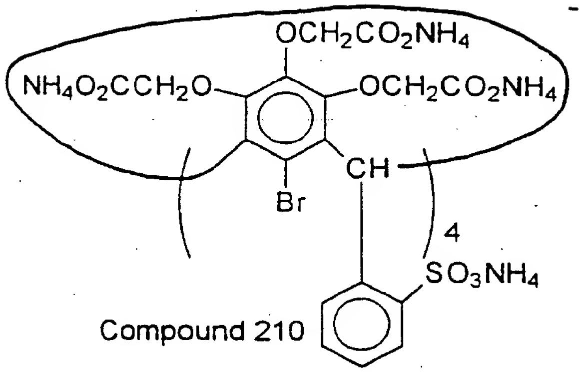

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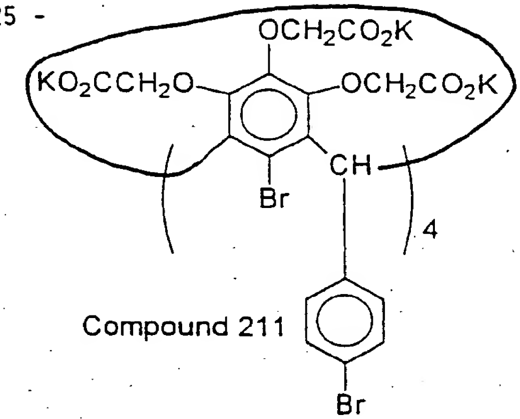

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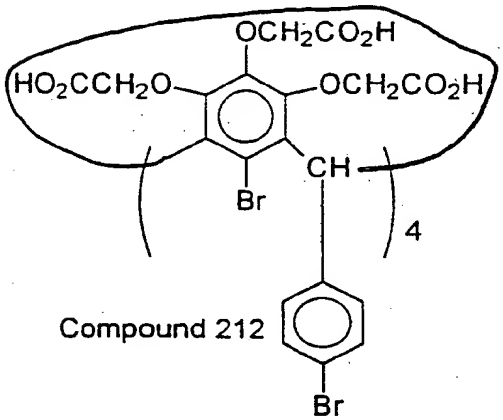

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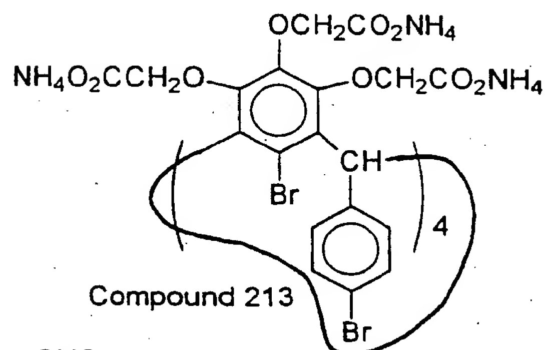

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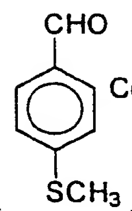

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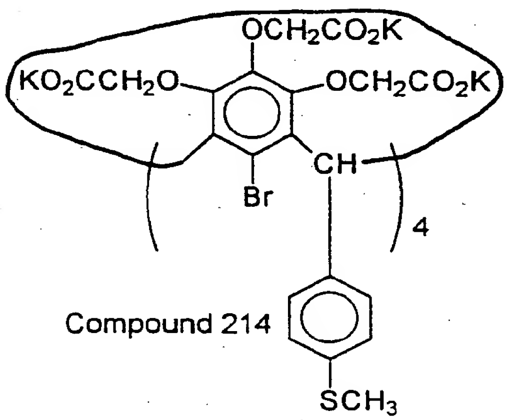

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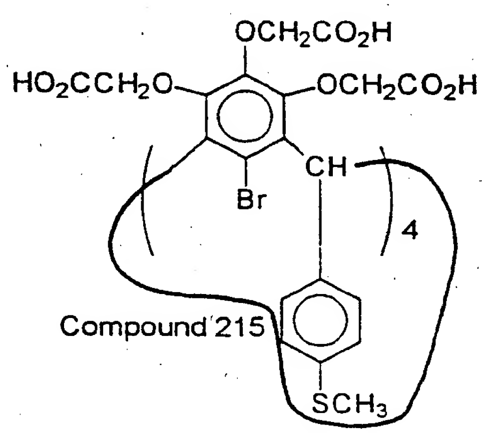

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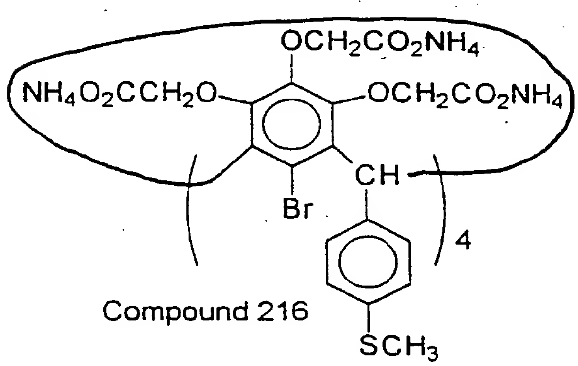

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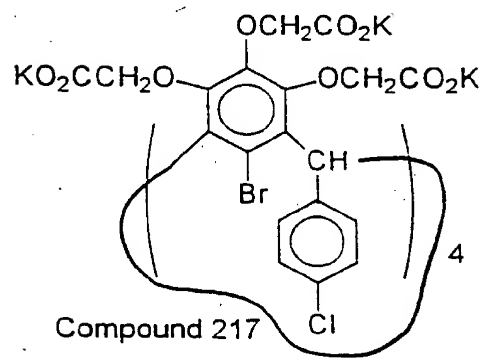

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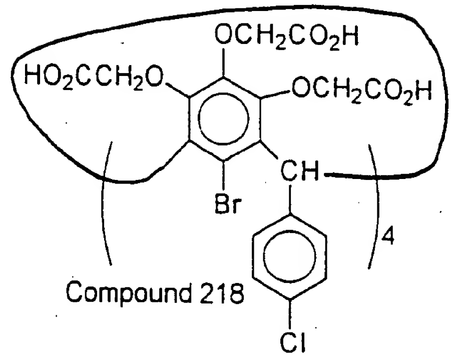

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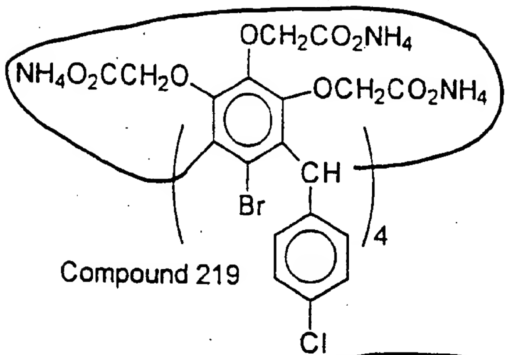

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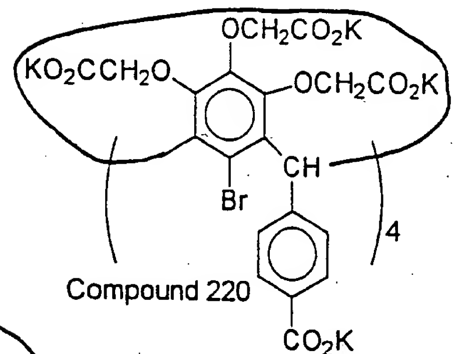

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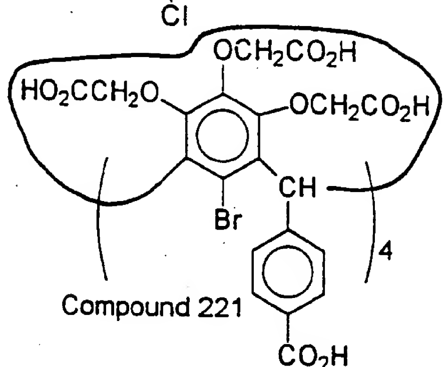

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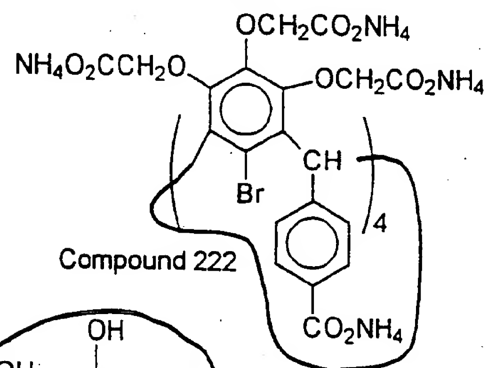

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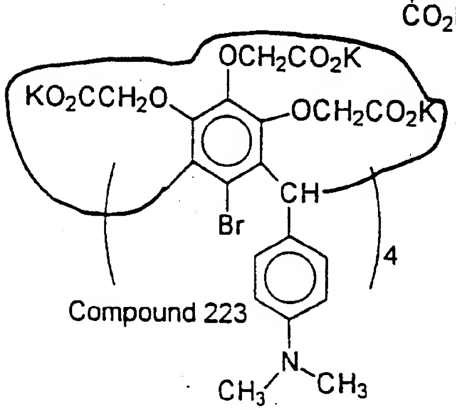

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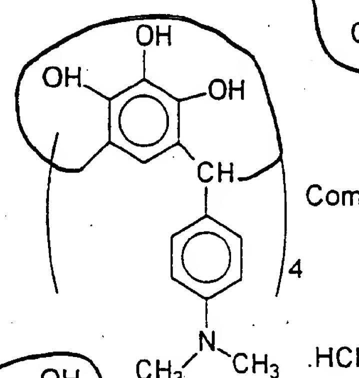

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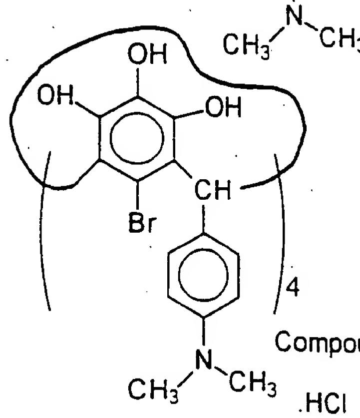

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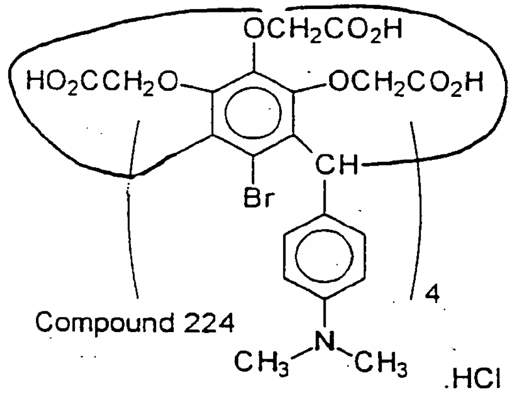

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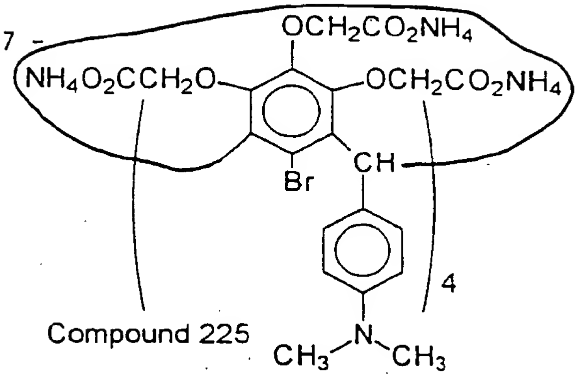

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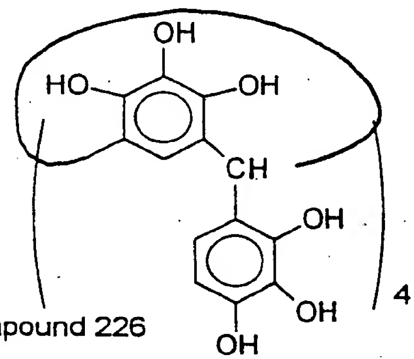

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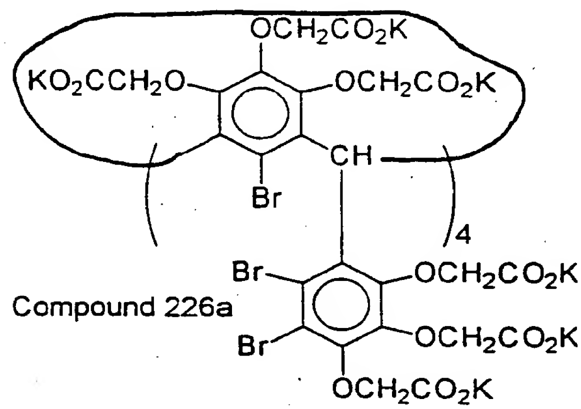

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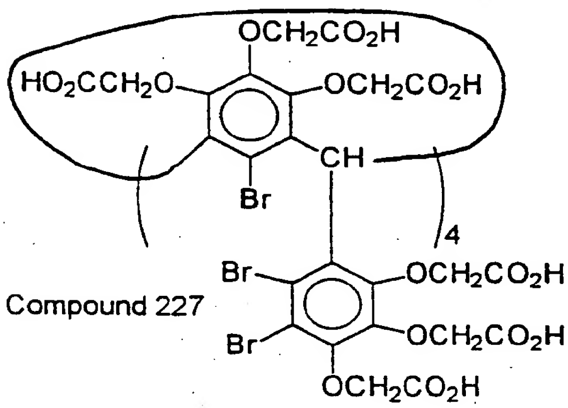

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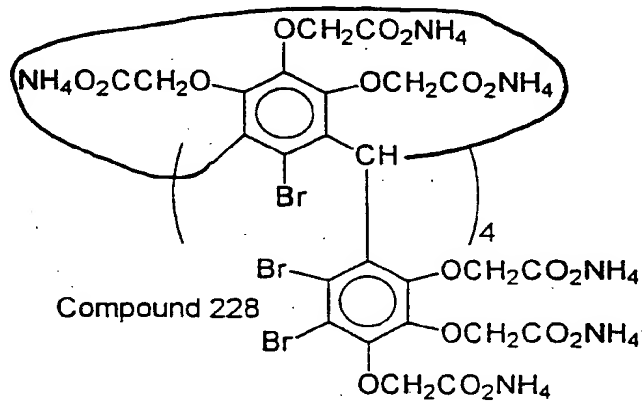

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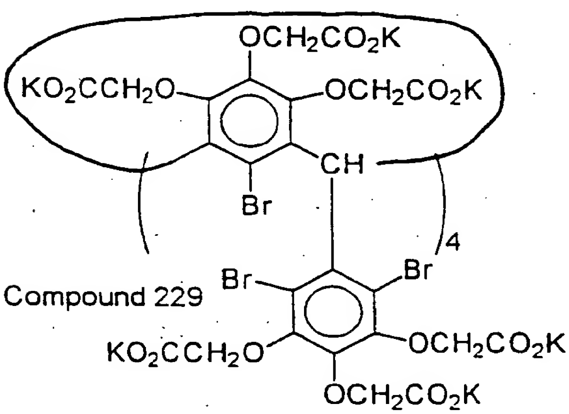

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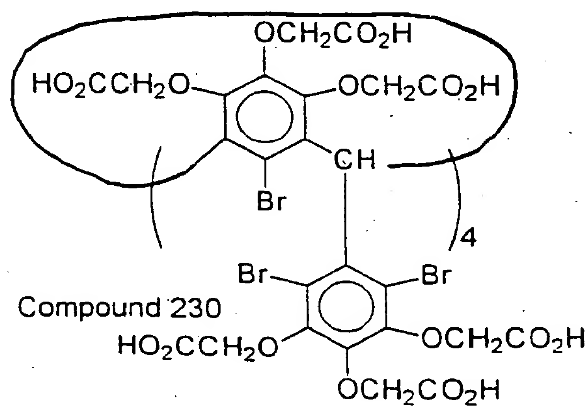

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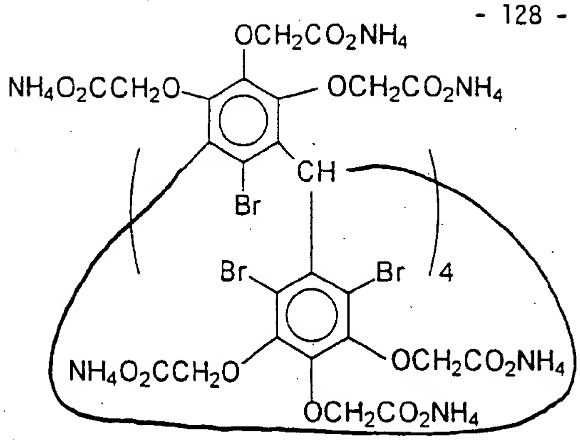

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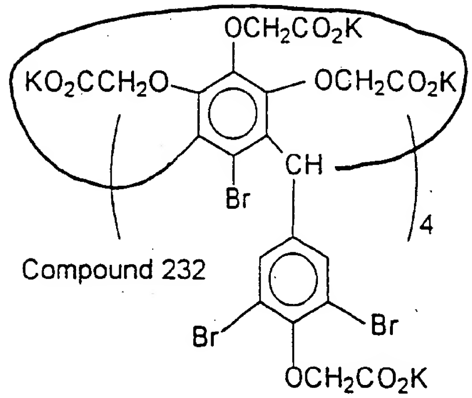

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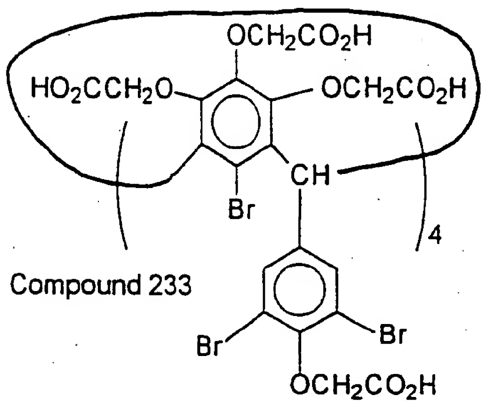

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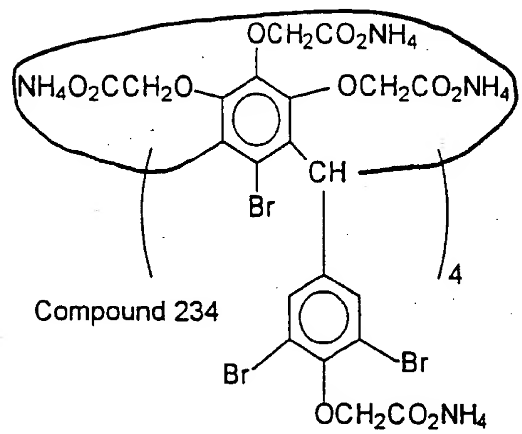

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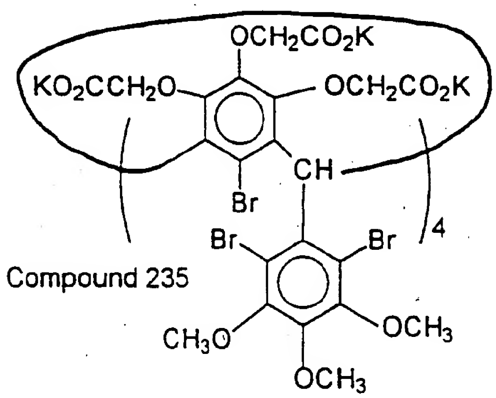

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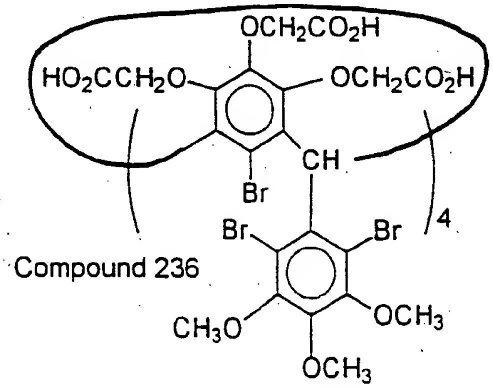
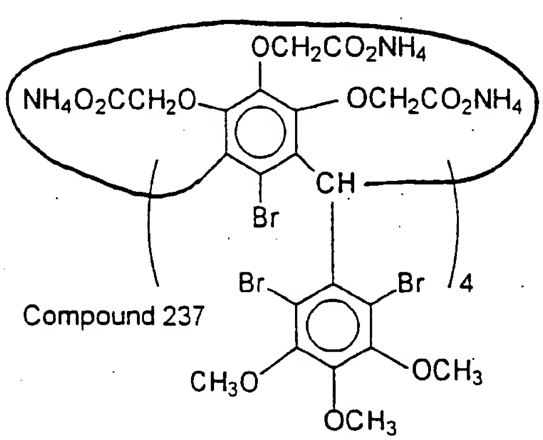
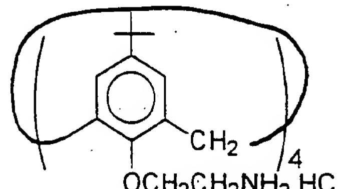


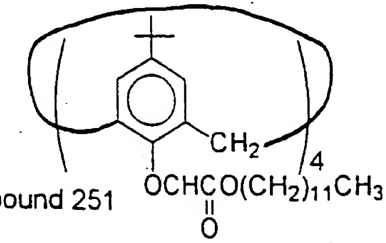
Compound 236



Compound 237



Compound 249



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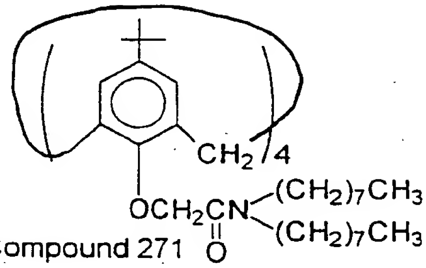

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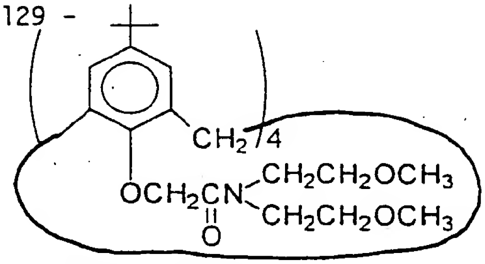

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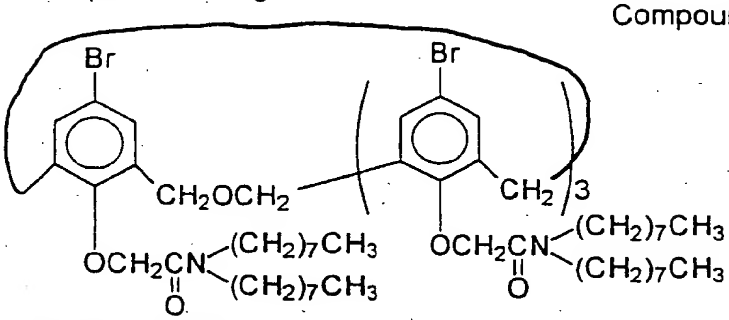

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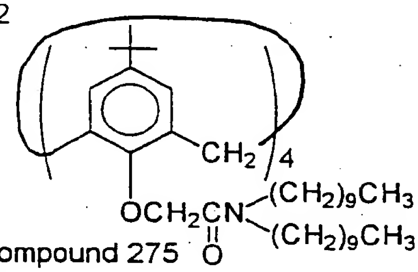
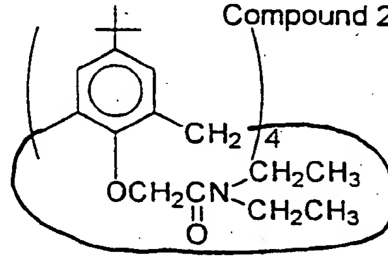
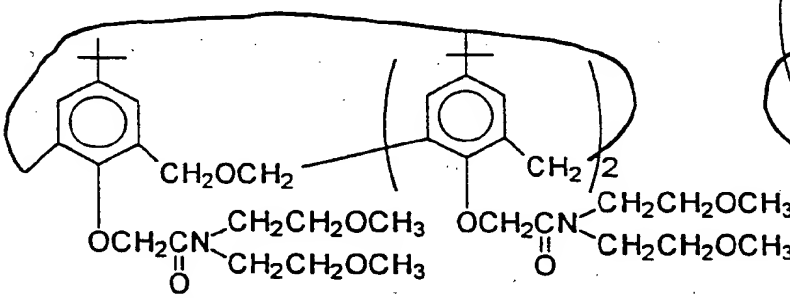


Compound 275

Compound 277





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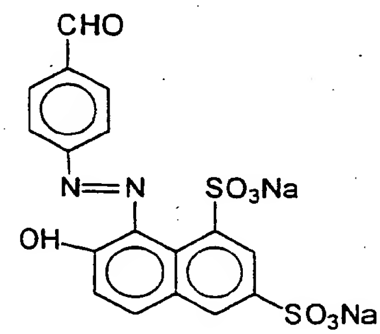
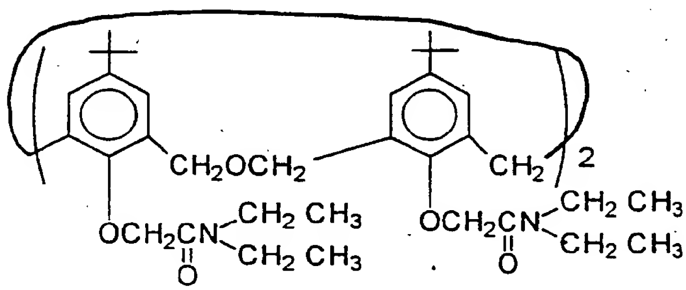


Compound 278a



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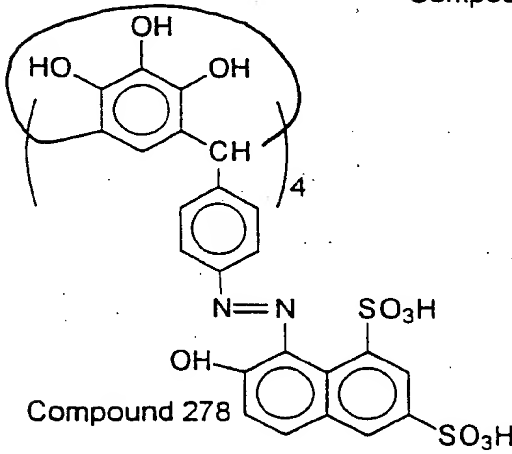
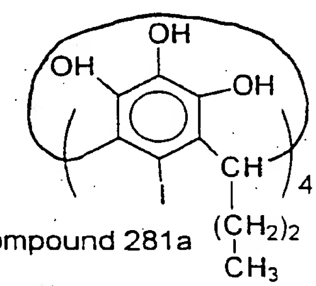
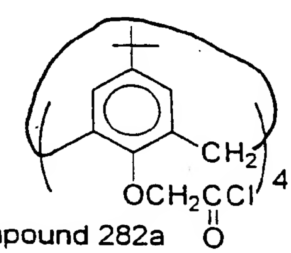


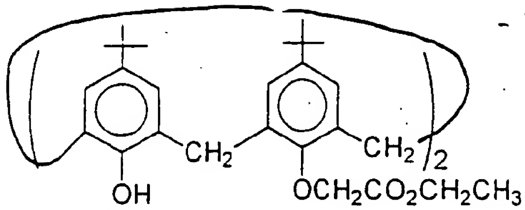
Compound 278



Compound 281a



Compound 282a



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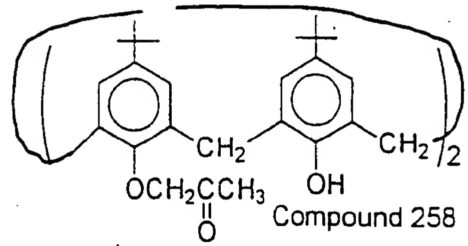

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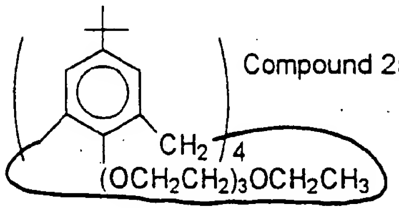

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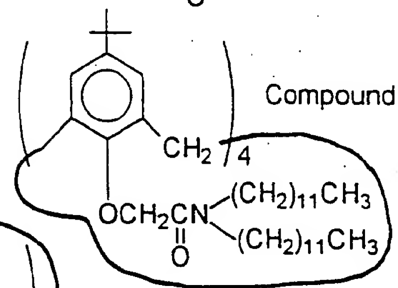
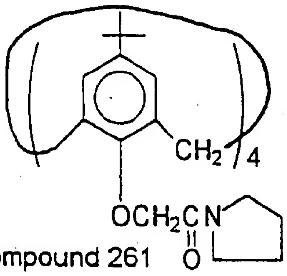
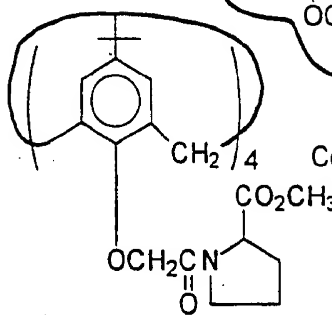


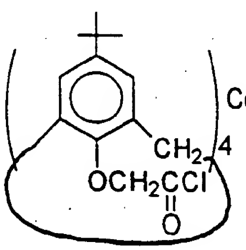
Compound 260



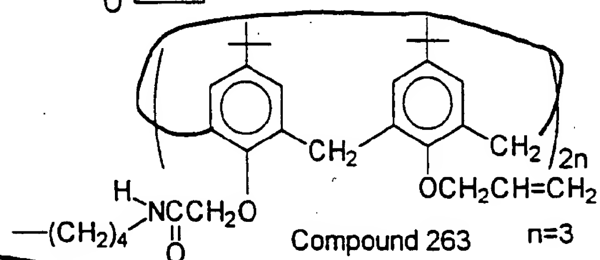
Compound 261



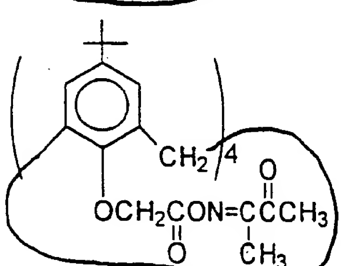
Compound 262



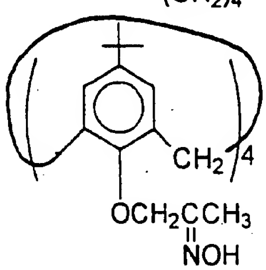
Compound 262a



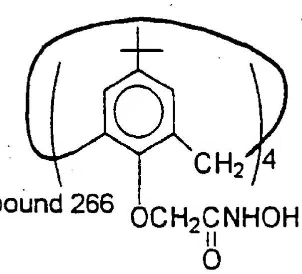
Compound 263 n=3



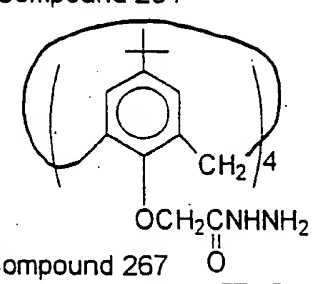
Compound 264



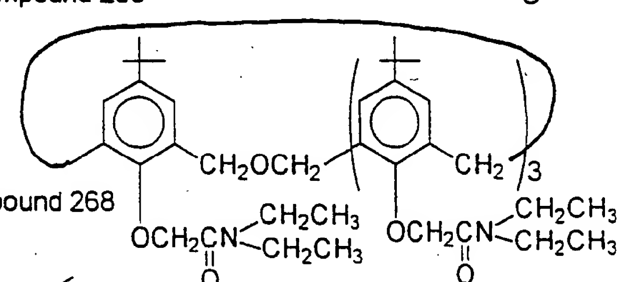
Compound 265



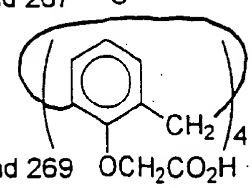
Compound 266



Compound 267



Compound 268



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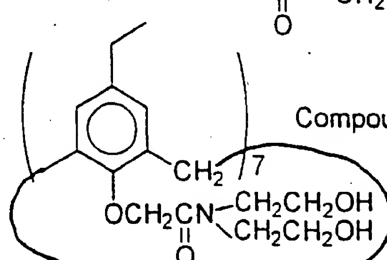

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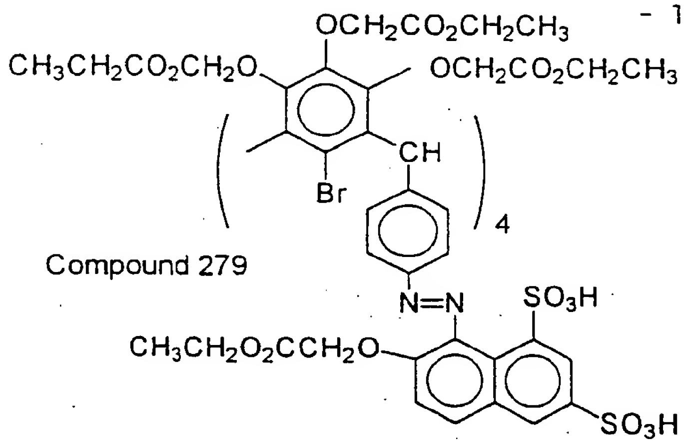

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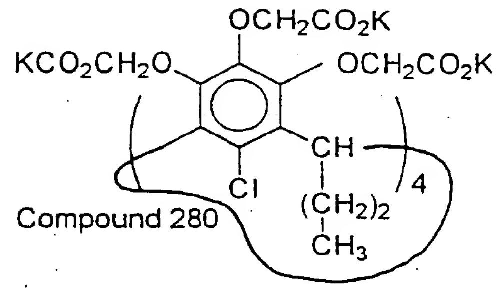
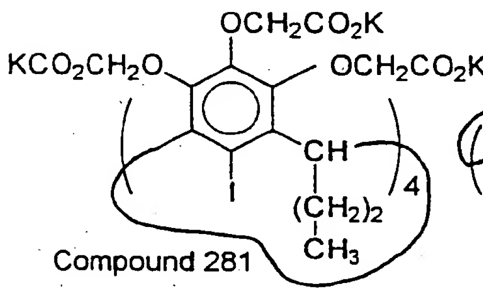
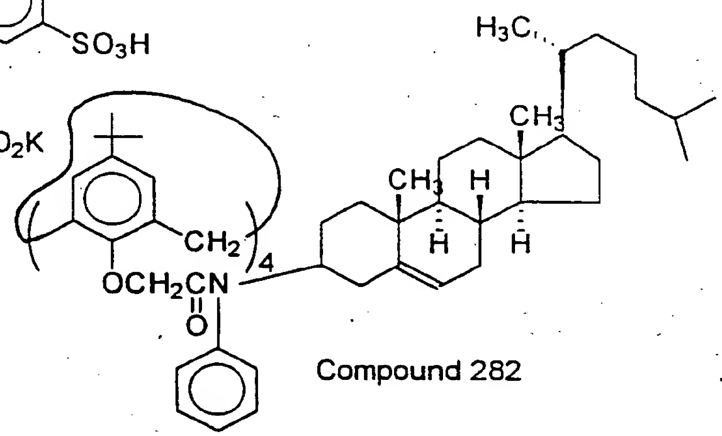


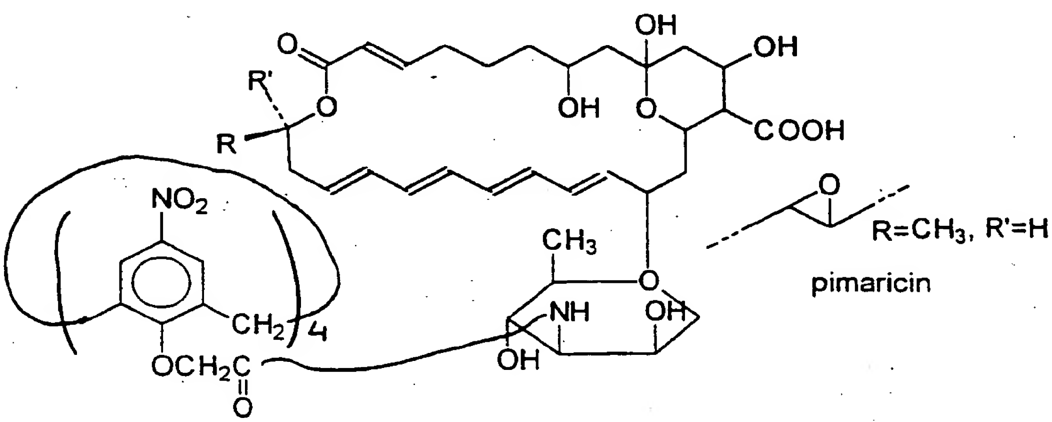
Compound 280



Compound 281



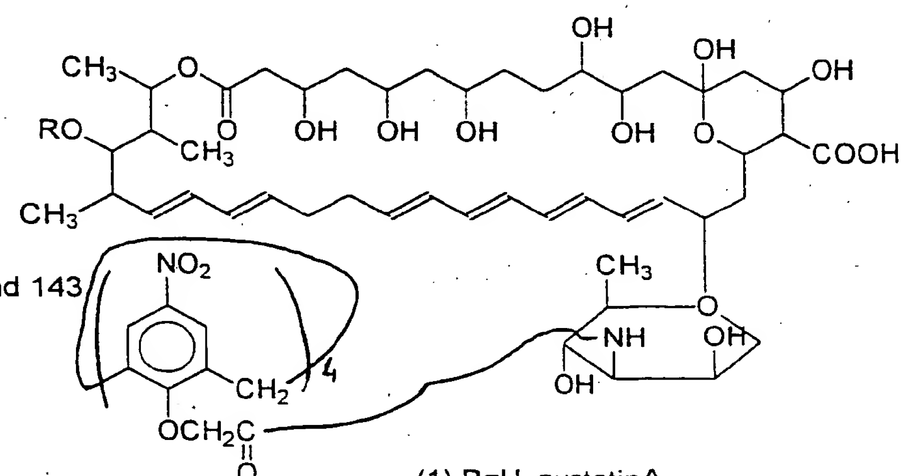
Compound 282



$R=CH_3$, $R'=H$

pimaricin

Compound 142



Compound 143

(1) R=H nystatinA_1

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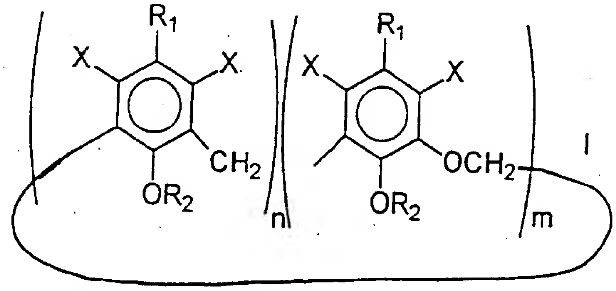
Claims

1. Calixarene or oxacalixarene derivatives selected from those of

(a) the formula I



I

wherein $n + m = 3 - 8$

$m = 0 - 3$

$n = 0 - 8$

R^1 is H, halogen, hydrocarbyl, aryl, hydrocarbylaryl or a substituted derivative thereof, NO_2, SO_3M where M is an alkali metal, SO_3H, $R^1 = OR^2$ where R^2 is

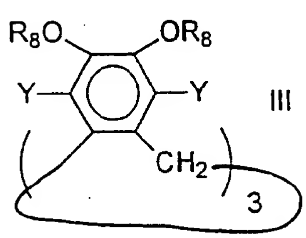
$$R^2 = CH_2\overset{O}{\overset{\|}{C}}\,OR^3 \quad \text{or} \quad CH_2\overset{O}{\overset{\|}{C}}O\frac{\underline{M}^P}{P} \quad \text{or} \quad CH_2\overset{O}{\overset{\|}{C}}N\begin{smallmatrix}R^4\\ R^5\end{smallmatrix}$$

X is halogen, NO_2, CO_2H, CN or other electron withdrawing group.

R^3 is alkyl or a substituted derivative thereof,

M is a metal or ammonium ion, P is the charge on the metal ion, R^4 or R^5 may be the same or different, or both may be part of amino acid ester or poly(amino acid ester) of one or more of the same or different amino acids or part of a cyclic polyene antibiotic/antifungal drug or part of a cyclic nitrogen heterocycle; or

(b)cyclotriveratrylene derivatives of formula III



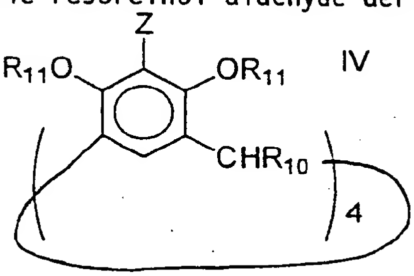
III

wherein Y is H, halogen, NO_2, but preferably halogen,

R^8 is H or $CH_2CO_2R^9$ or $CH_2CO_2\frac{\underline{M}^P}{P}$, preferably CH_2CO_2K,

R^9 is alkyl, aryl, alkaryl or a substituted derivative thereof, M is metal ion, preferably an alkaline metal or alkaline earth metal, and p is the charge on the metal ion; or

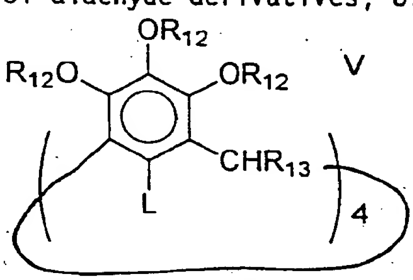
(c) cyclic tetrameric resorcinol-aldehyde derivatives of formula IV



IV

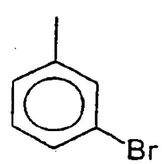
wherein R^{11} is the same as R^8 defined above, Z is halogen or nitro, preferably halogen, R^{10} is alkyl, aryl, alkaryl or a substituted derivative thereof; or

(d) cyclic tetrameric pyrogallol-aldehyde derivatives, of formula V



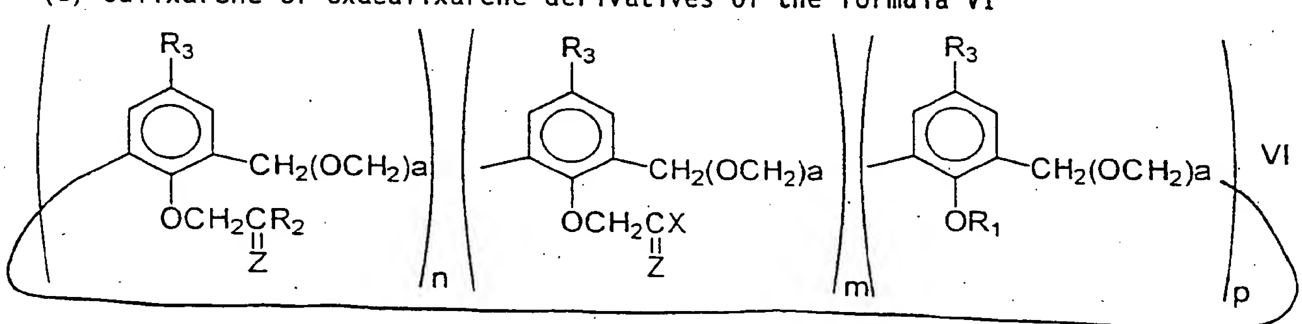
V

wherein R^{12} is the same as R^8 defined above, preferably $CH_2CO_2NH_4$, CH_2CO_2K or CH_2CO_2M where M is defined above, L is H, halogen (preferably bromine) or nitro, or other electron withdrawing group, R^{13} is alkyl, aryl, alkaryl or a substituted derivative thereof preferably $(CH_2)_2CH_3$ or



; or

(e) calixarene or oxacalixarene derivatives of the formula VI



VI

wherein

$n + m + p = 3\text{-}8$

$m = 0\text{-}8$

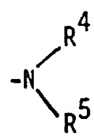
$n = 0\text{-}8$

$p = 0\text{-}8$

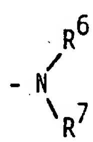
a, which may be the same or different on each aryl group, is 0 or 1; with the proviso that when a is 1 in more than one aryl group, the methylene and ether bridges may or may not alternate within the oxacalixarene molecule,

R', which may be the same or different on each aryl group, is -H or hydrocarbyl, aminohydrocarbylaryl, hydrocarbylaryl or a substituted derivative or salt thereof;

R^2, which may be the same or different on each aryl group, is hydrocarbyl, aryl, hydrocarbylaryl, hydrocarbyloxy, aryloxy, hydrocarbylaryloxy, alicyclic, hydrocarbylthio, arylithio, hydrocarbylarylthio, oxime, or a substituted derivative thereof;

or 

wherein R^4 and R^5, which may be the same or different, are -H or hydrocarbyl, aryl, hydrocarbylaryl or a substituted derivative thereof and R^4 and R^5 may form a cycloaliphatic ring, which may in turn be substituted; or R^5 may be



wherein R^6 and R^7, which may be the same or different are H or hydrocarbyl, aryl, hydrocarbylaryl or a substituted derivative thereof; or R^5 is OR^4 wherein R^4 is as defined above; or R^5 is a residue of a hydrocarbyl, aryl or hydrocarbylaryl group or of a substituted derivative thereof providing bond to another calixarene or oxacalixarene derivative wherein R^5 is a similar residue;

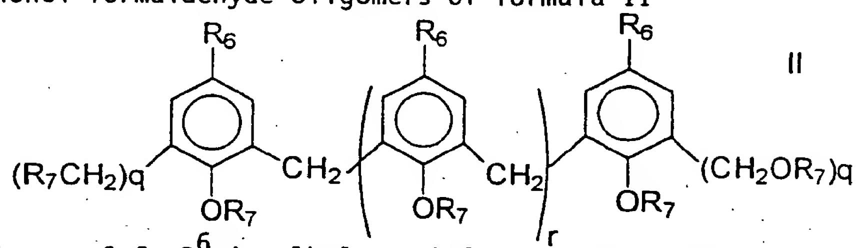
R^3, which may be the same or different on each aryl group, is -H, halogen, hydrocarbyl, aryl, hydrocarbylaryl or a substituted derivative thereof;

X is -OH, -OM (wherein M is a salt forming metal), or a group containing an acrylate or methacrylate functional group;

Z is O or S or NOH;

or a polymer of a compound of the formula VI in which X is a group containing an acrylate or methacrylate functional group.

2. Acyclic phenol-formaldehyde oligomers of formula II



II

wherein q = 0-1, r = 0-6; R^6 is alkyl, H, halogen, aryl, alkaryl or a substituted derivative thereof,

R^7 is H or $CH_2CO_2\underline{M}^p_p$ where p is the charge on the metal ion, and

M is a metal ion.

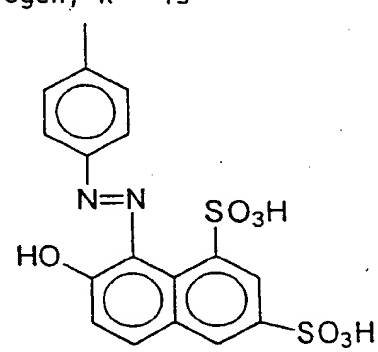
3. A compound as claimed in claim 1 in which in compounds of Formula I R^1 is NO_2 or a halogen, particularly bromine,

R^3 is $CH_2CH_2OCH_3$ when R^1 is ethyl, n is 7 and m is 0,

M is an alkali metal, alkaline earth metal, ammonium or substituted derivative thereof, R^2 is preferably CH_2CO_2K or $CH_2CO_2NH_4$, and the cyclic polyene drug is selected from Amphotericin B, a lactam antibiotic particularly a penicillin derivative or the aminoglucoside sinefungin, and the cyclic nitrogen heterocycle is selected from an aminotetrazole or aminotriazole.

4. A compound as claimed in Claim 2 wherein in compounds of Formula II R^6 is halogen, M is an alkaline metal or alkaline earth metal, R^7 is preferably CH_2CO_2K.

5. A compound of Formula V as claimed in Claim 1 wherein when R^{12} is hydrogen and L is hydrogen, R^{13} is



6. Antifungal agents of formula I as claimed in Claim 1 selected from those
(i) wherein m is at least 1 and X is -OH or -OM,
preferably n = p = o,
m is 4-8 and X is -OK, or OH
most preferably wherein m is 7 or 8;
(ii) m = n = o and R' is $CH_2CH_2NH_2$ HCl;
(iii) R^2 is a pyrrole group;
(iv) Z is NOH; and
(v) m = p = o, n = 4, and a = o.

7. An antifungal agent as claimed in claim 6 wherein if X is a group containing an acrylate or methacrylate functional group, said group is preferably of the formula

$$-O(CH_2)_q\,O\,\underset{\underset{O}{\|}}{C}\,\underset{\underset{R''}{|}}{C} = CH_2$$

wherein q = an integer 2-10 and
R" is H or CH_3.

8. A method for the synthesis of compounds of formula V defined in Claim 1 wherein pyrogallol is condensed with an aldehyde, the resulting tetramer optionally being halogenated.

9. A method as claimed in Claim 8 wherein the tetramer is converted to its alkyl acetate derivative and optionally base - hydrolysed to give a potassium acetate derivative, which may optionally further be converted to the acid and subsequently to the ammonium salt.

10. A method as claimed in Claim 9 wherein pyrogallol is condensed with an aldehyde in equimolar quantities in refluxing 37% aqueous HCl/ethanol 1/4 by volume, the precipitated tetramer washed with a minimum quantity of cold ethanol then brominated with one equivalent of bromine in $CHCl_3$ then converted to its ethyl acetate derivative by treatment with 24 equivalents of ethyl bromoacetate and 18 equivalents of anhydrous potassium carbonate in refluxing dry acetone, for 48 hours following which the unpurified ester is hydrolysed with an equal weight of potassium hydroxide in refluxing ethanol for 2 hours to give the potassium acetate derivative which is filtered off under nitrogen and washed with a minimum quantity of cold ethanol, conversion to acid being accomplished by addition of a minimum quantity of 37% aqueous

HCl and washing the precipitate with a minimum quantity of cold water, and conversion to the ammonium salt is by addition of excess 25% aqueous analar NH_4OH at 50°C in an oven overnight.

11. Anti-bacterial, anti-fungal, anti-viral and anti-cancer agents comprising as active ingredient calixarene or oxacalixarene derivatives of formulae I, II, III, IV, V or VI, as defined in Claims 1 or 4.

12. Pharmaceutical compositions comprising a pharmaceutically effective amount of any of the, either singly or in combination.

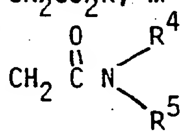
13. Use of any of the compounds claimed in claim 1 to 7, either singly or in combination, in the preparation of a medicament for the treatment of bacterial infection, fungal infection or viral infection, particularly HIV-1, HIV-2 or SIV infection.

14. A method of medical treatment comprising administering a therapeutically effective amount of any of the compounds claimed in Claims 1 to 7 to a patient, either singly or in combination.

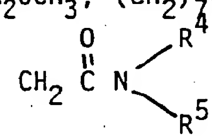
15. Compounds as claimed in Claim 1, with antibiotic activity, wherein R^2 is CH_2CO_2H, m is 0, n is 4, X is hydrogen and R^1 is hydrogen.

16. Compounds as claimed in Claim 1 selected from those wherein

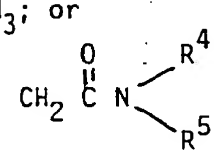
(a) R^2 is CH_2CO_2K, m is 0, n is 5, X is hydrogen and R^1 is Br; or

(b) R^2 is 

n is 4, m is 0, X is hydrogen, R^1 is t-butyl and R^4 is the same as R^5 and is $CH_2CH_2OCH_3$, $(CH_2)_7CH_3$, $(CH_2)_9CH_3$; or 

(c) R^2 is 

n is 3, m is 1, X is hydrogen, R^1 is t-butyl, and R^4 is the same as R^5 and is CH_2CH_3; or

(d) R^2 is 

n is 3, m is 1, X is hydrogen, R^1 is Br and R^4 is the same as R^5 and is $(CH_2)_7CH_3$; or

(e) R^2 is $CH_2\overset{O}{\overset{\|}{C}}N\genfrac{}{}{0pt}{}{R^4}{R^5}$

n is 2, m is 2, X is hydrogen, R^1 is t-butyl and R^4 is the same as R^5 and is $CH_2CH_2OCH_3$;

or

(f) R^2 is $CH_2\overset{O}{\overset{\|}{C}}N\genfrac{}{}{0pt}{}{R^4}{R^5}$

n is 7, X is hydrogen, R^1 is ethyl and R_4 is the same as R^5 and is CH_2CH_2OH.

17. Compounds as claimed in claim 1, with anti-cancer activity, wherein

(a) R^1 is CH_2CH_3, R^2 is $OCH_2\overset{O}{\overset{\|}{C}}\text{-}OCH_2CH_2O(CH_2)_3CH_3$, n is 7, m is 0 and X is hydrogen; or

(b) R^1 is t-butyl, n is 4, m is 0, X is hydrogen, R^2 is

$OCH_2\overset{O}{\overset{\|}{C}}N\genfrac{}{}{0pt}{}{R^4}{R^5}$

where R^4 is the same as R^5 and is CH_2CH_3, $CH_2CH_2OCH_3$; or

(c) R^1 is t-butyl, n is 5, m is 0, X is hydrogen, R^2 is

$OCH_2\overset{O}{\overset{\|}{C}}N\genfrac{}{}{0pt}{}{R^4}{R^5}$

where R^4 is the same as R^5 and is CH_2CH_3; or

(d) R^1 is t-butyl, n is 2, m is 2, X is hydrogen, R^2 is

$OCH_2\overset{O}{\overset{\|}{C}}N\genfrac{}{}{0pt}{}{R^4}{R^5}$

where R^4 is the same as R^5 and is CH_2CH_3,

(e) R^1 is t-butyl, n is 4, m is 0,
R^4 is phenyl and R^5 is cholesterol.

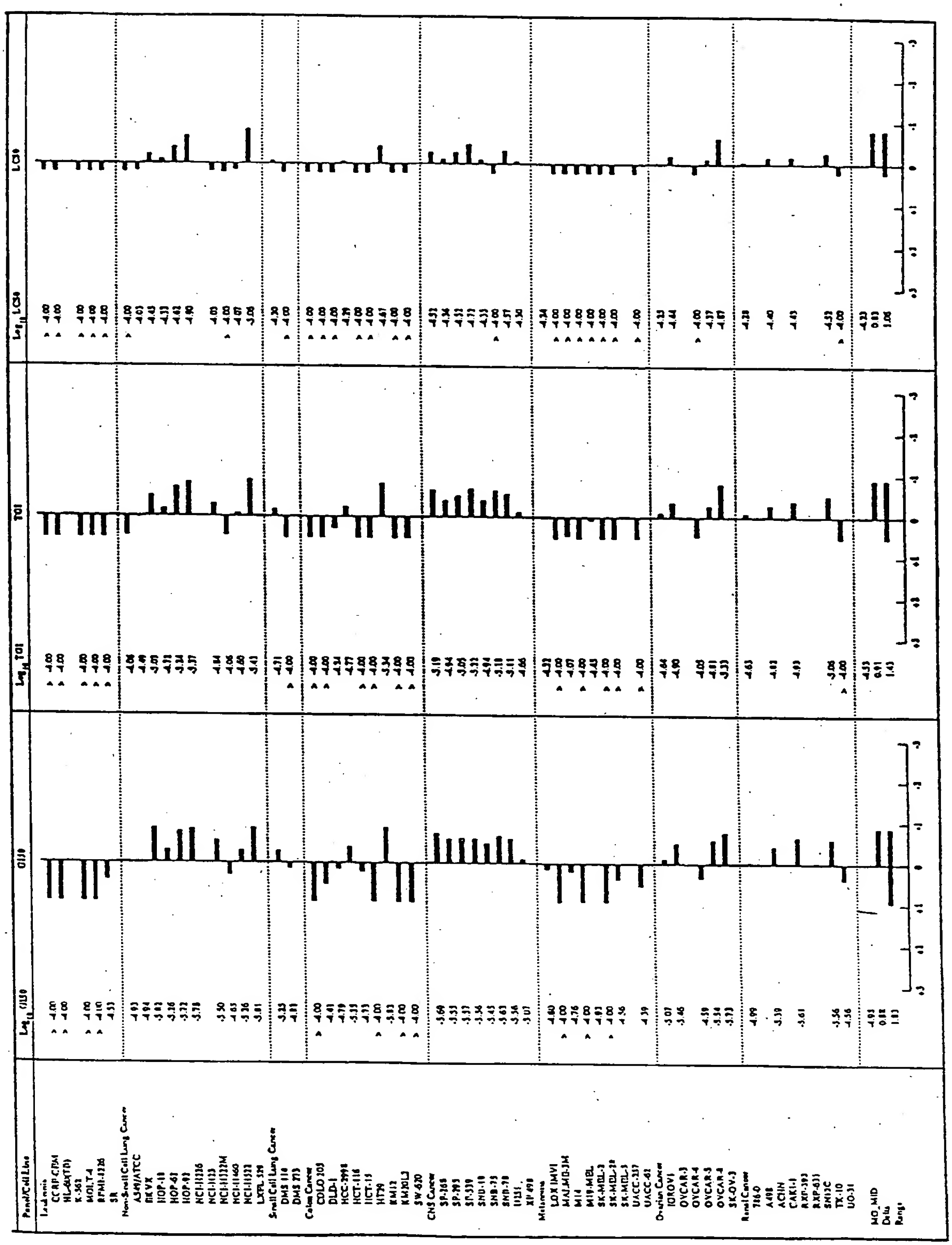


Fig. 1

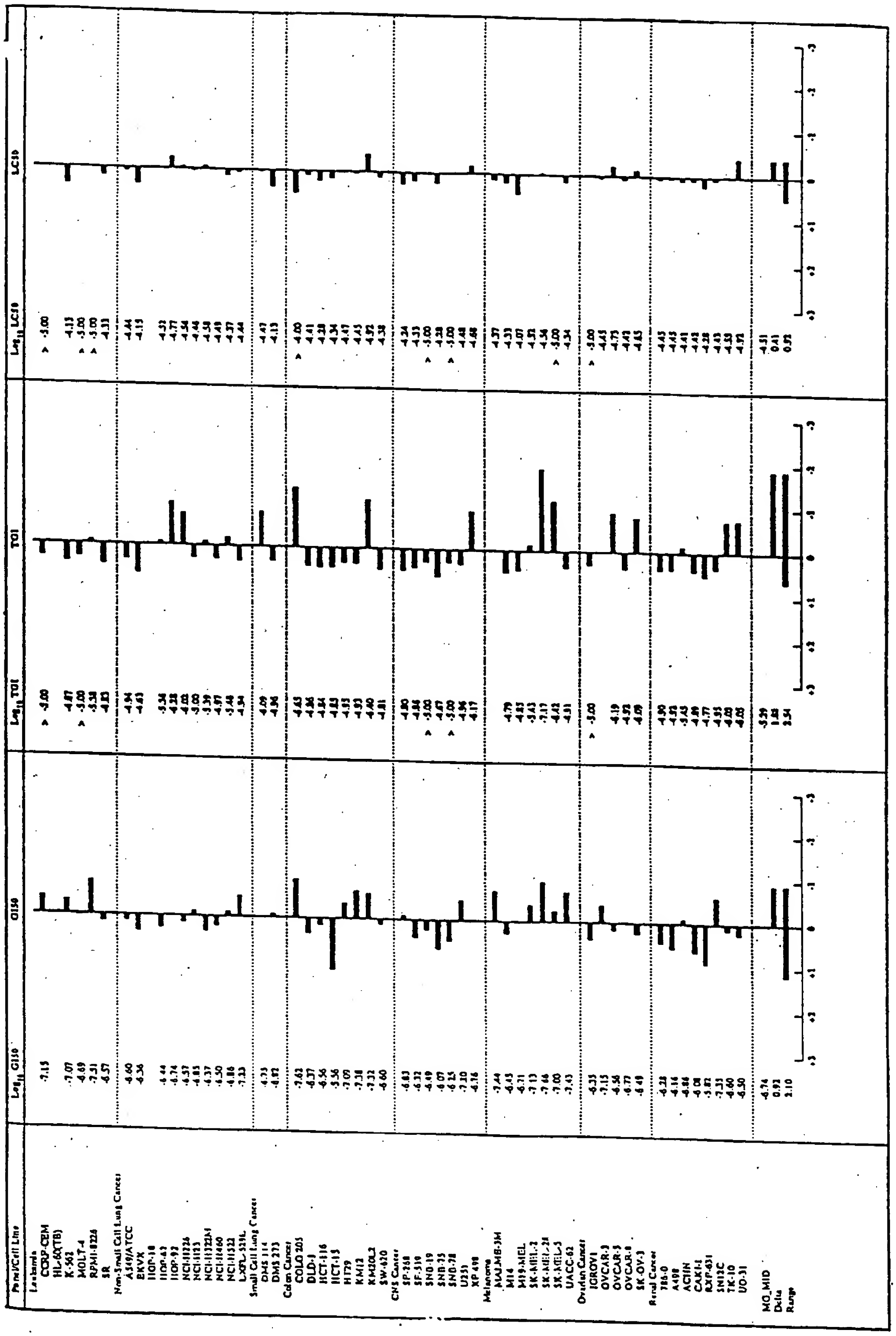


Fig. 2

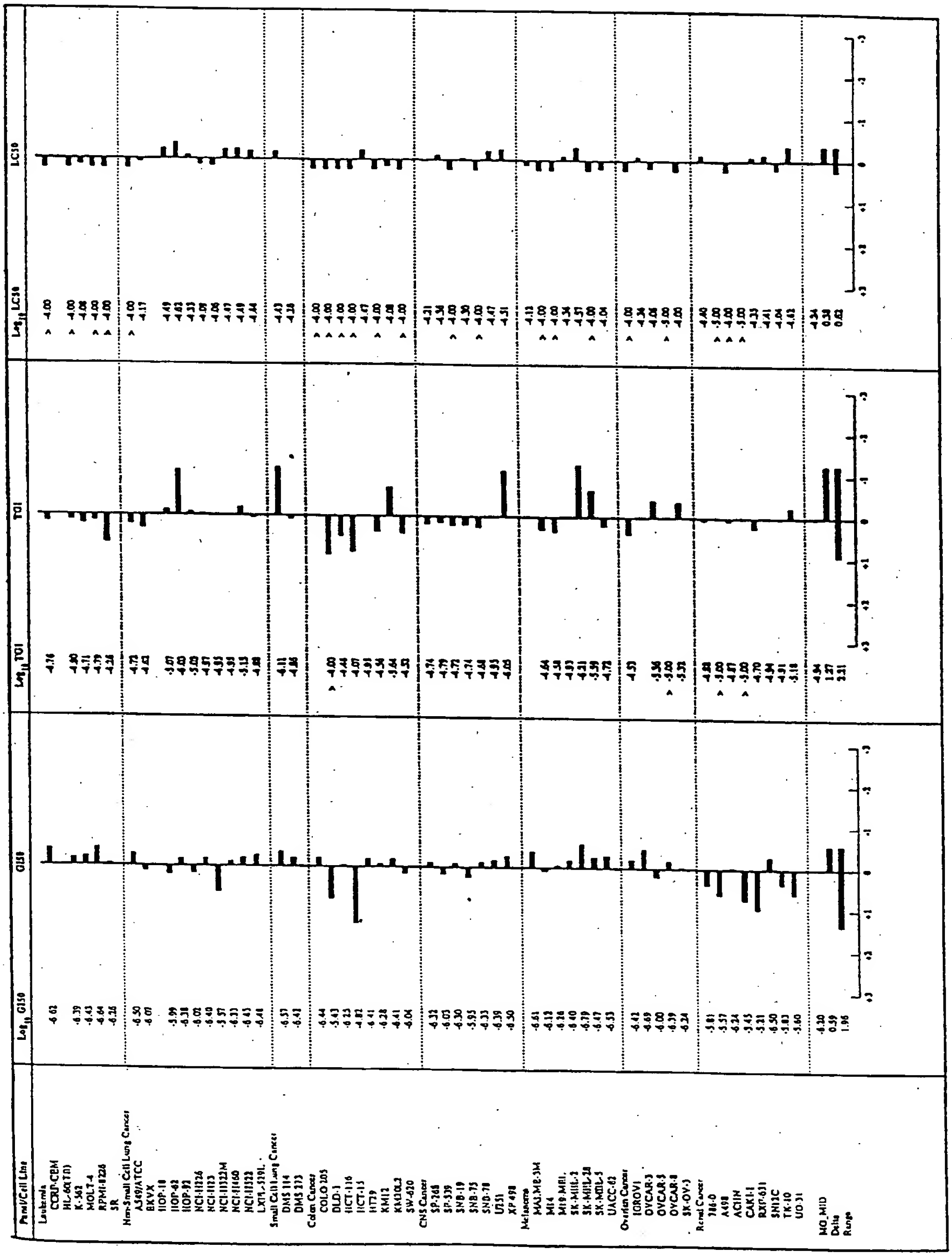


Fig. 3

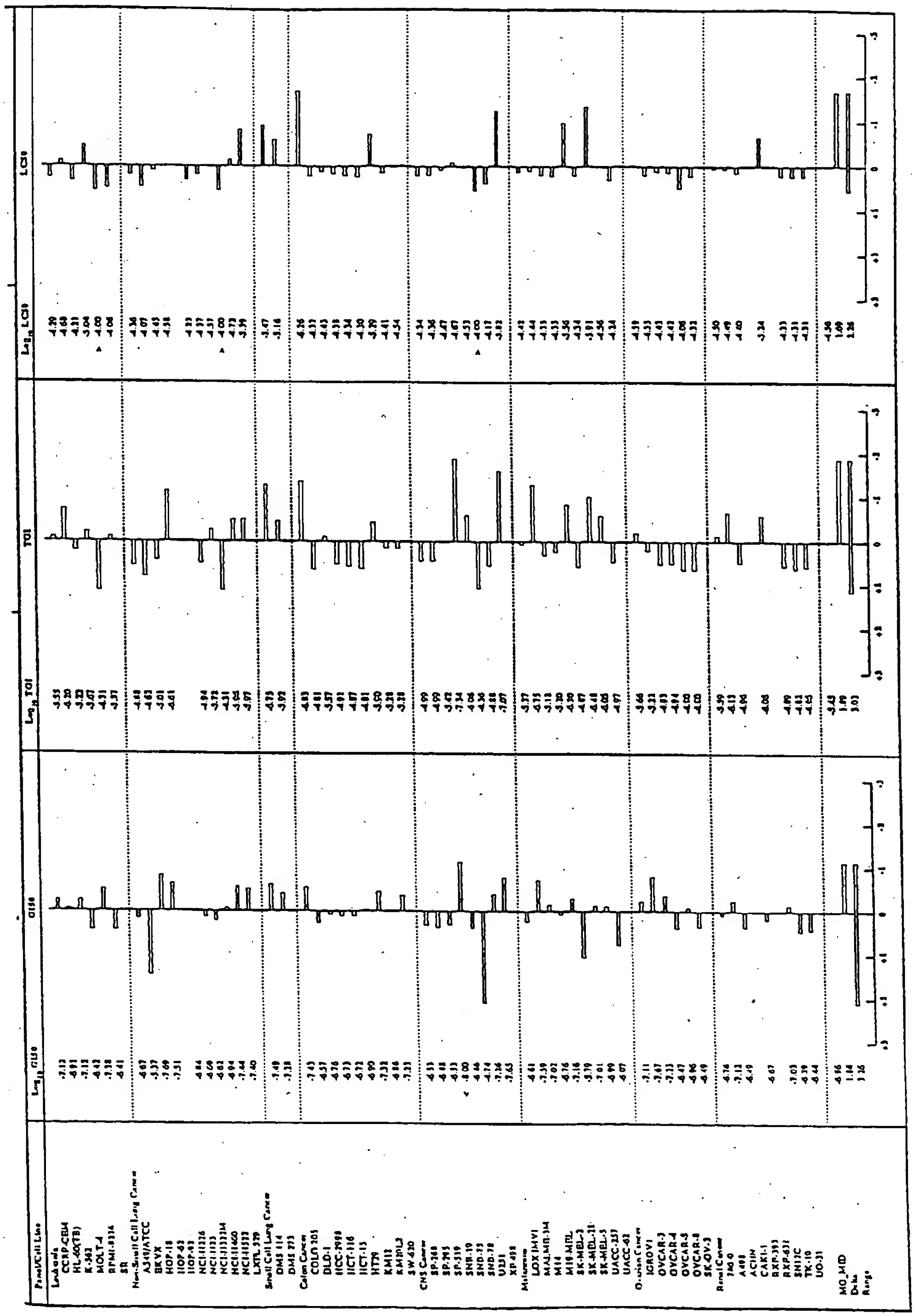


Fig. 4.

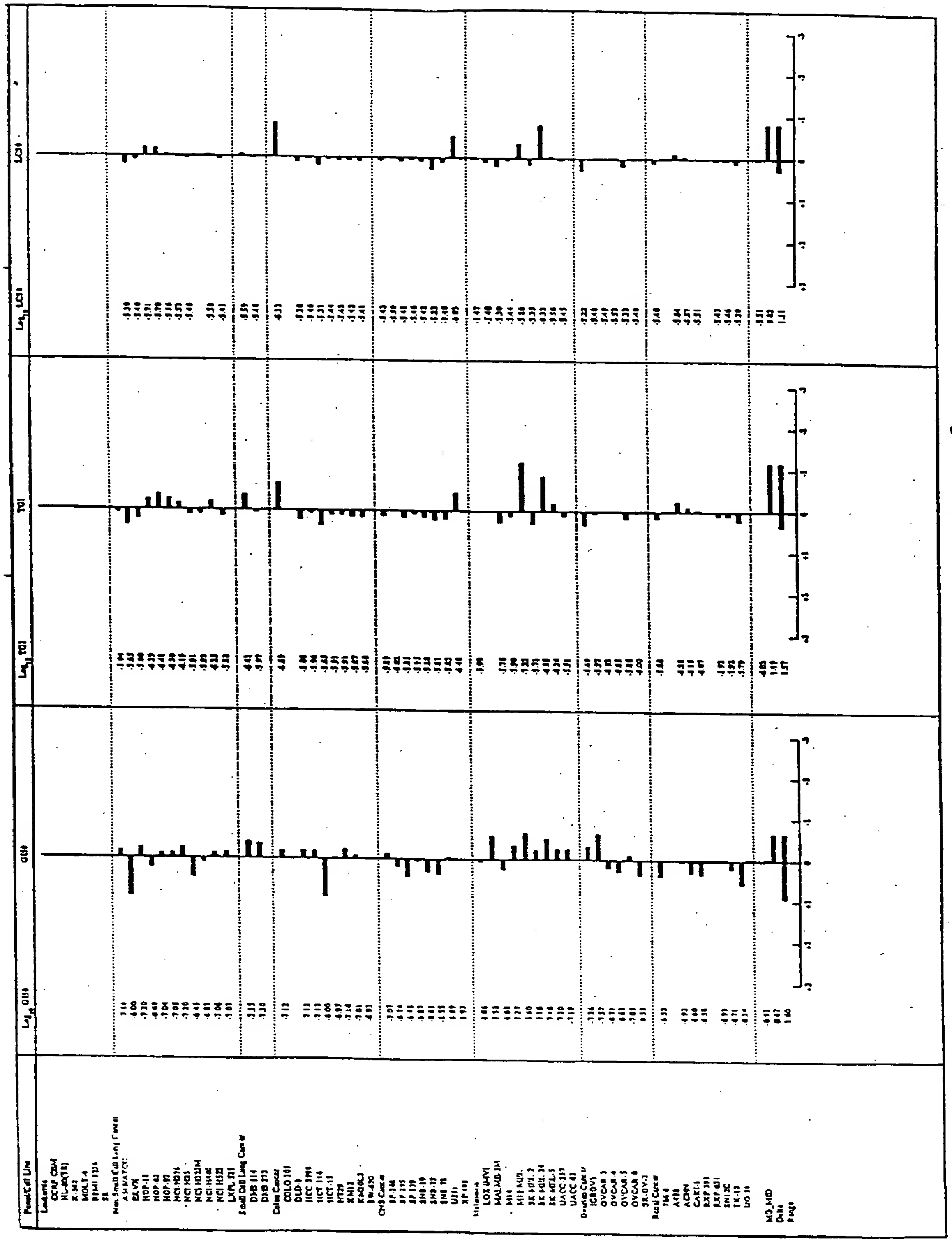
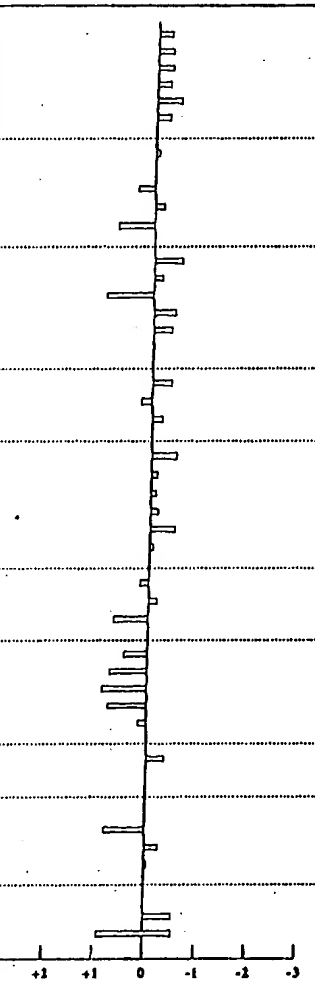
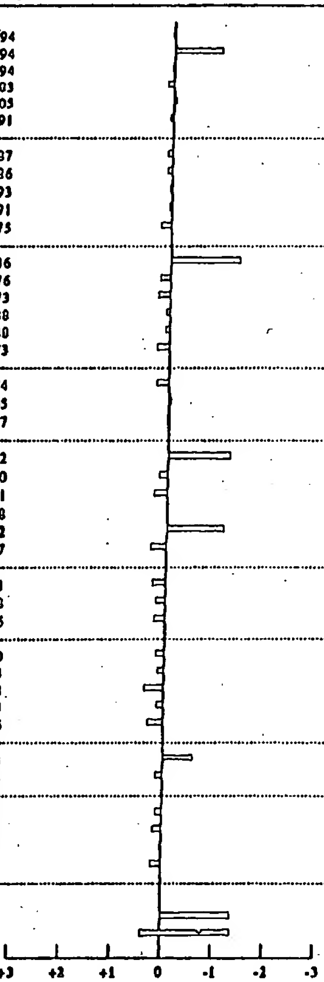


Fig. 5.

Panel/Cell Line	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
Leukemia			
CCRF-CEM	-7.45	-5.94	-5.19
HL-60(TB)	-7.48	-6.94	-6.10
K-562	-7.47	-5.94	-5.30
MOLT-4	-7.44	-5.03	-5.23
RPMI-8226	-7.66	-6.05	-5.17
SR	-7.45	-5.91	> -5.00
Non-Small Cell Lung Cancer			
A549/ATCC	-7.25	-5.87	-5.39
EKVX	-7.16	-5.86	-5.41
HOP-62	-6.80	-5.93	-5.45
NCI-H23	-7.36	-5.91	-5.23
NCI-H322M	-6.43	-5.75	-5.36
Colon Cancer			
COLO 205	-7.73	-7.36	-6.90
HCC-2998	-7.35	-5.76	-5.37
HCT-15	-6.22	-5.73	-5.36
HT29	-7.62	-5.88	-5.39
KM12	-7.56	-5.80	-5.32
SW-620	-7.18	-5.73	-5.20
CNS Cancer			
SF-268	-7.57	-5.74	-5.17
SF-295	-6.95	-6.05	-5.38
SNB-75	-7.40	-5.97	-5.48
Melanoma			
MALME-3M	-7.67	-7.22	-5.84
M14	-7.31	-5.80	-5.30
SK-MEL-28	-7.28	-5.71	-5.31
SK-MEL-5	-7.34	-5.98	-5.49
UACC-257	-7.67	-7.12	-5.46
UACC-62	-7.24	-5.67	-5.20
Ovarian Cancer			
IGROV1	-6.97	-5.71	-5.20
OVCAR-8	-7.33	-5.78	-5.03
SK-OV-3	-6.46	-5.76	-5.34
Renal Cancer			
786-0	-6.67	-5.80	-5.38
CAKI-1	-6.40	-5.84	-5.42
RXF-393	-6.25	-5.58	-5.11
SN12C	-6.37	-5.83	-5.40
TK-10	-6.97	-5.66	-5.31
Prostate Cancer			
PC-3	-7.53	-6.58	-5.56
DU-145	-7.15	-5.83	-5.38
Breast Cancer			
MCF7	-7.17	-5.84	-5.29
MDA-MB-231/ATCC	-6.34	-5.79	-5.38
MDA-N	-7.44	-5.95	-5.45
T-47D	-7.21	-5.77	-5.17
MG_MID	-7.16	-5.98	-5.39
Delta	0.58	1.38	1.52
Range	1.51	1.79	1.90





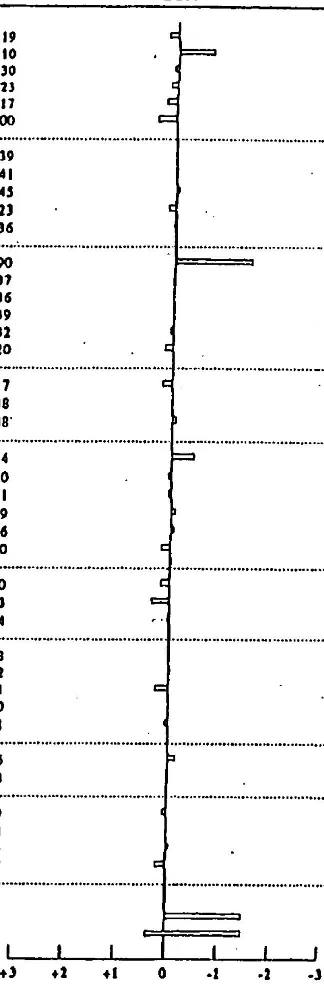


Fig. 6

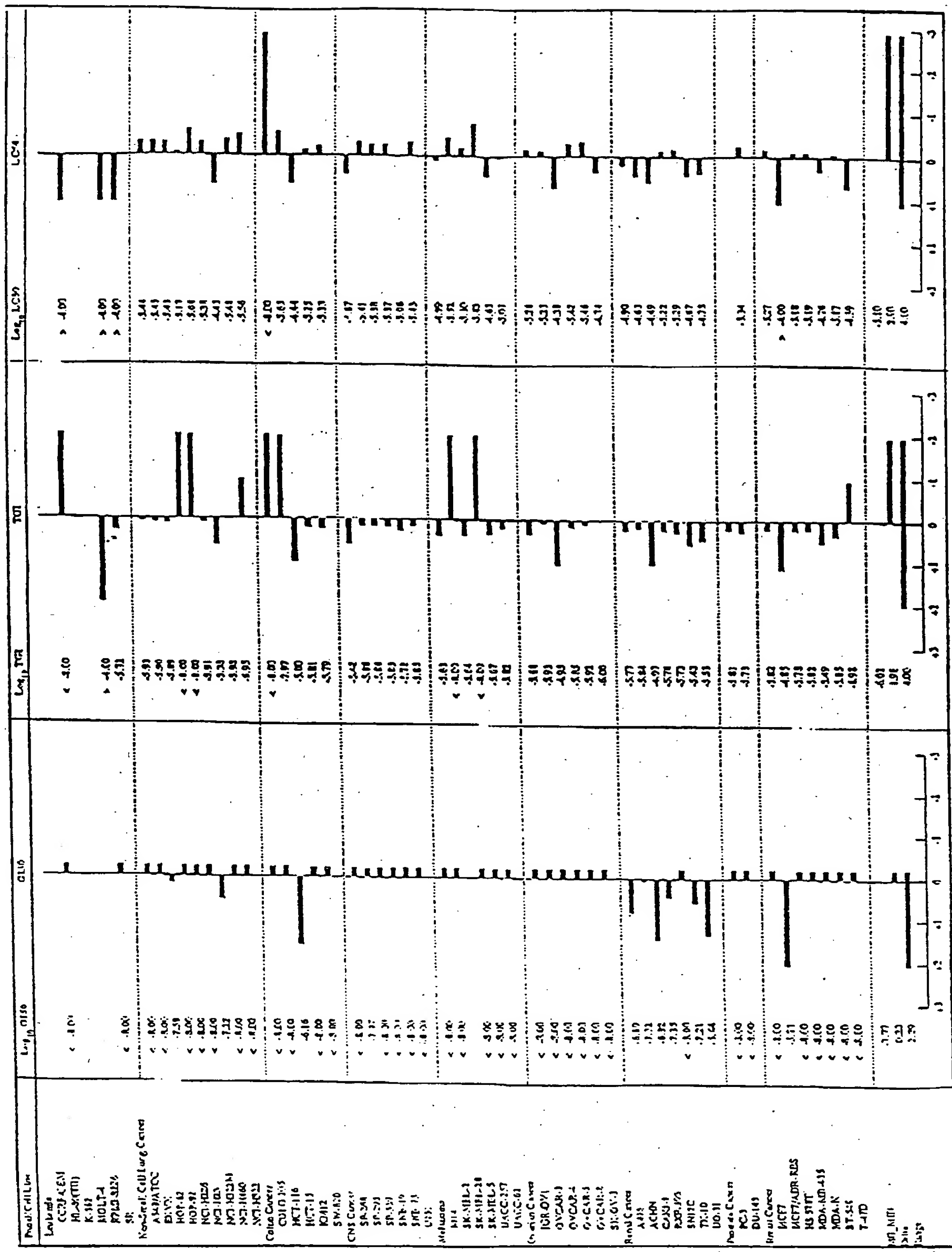


Fig. 7.

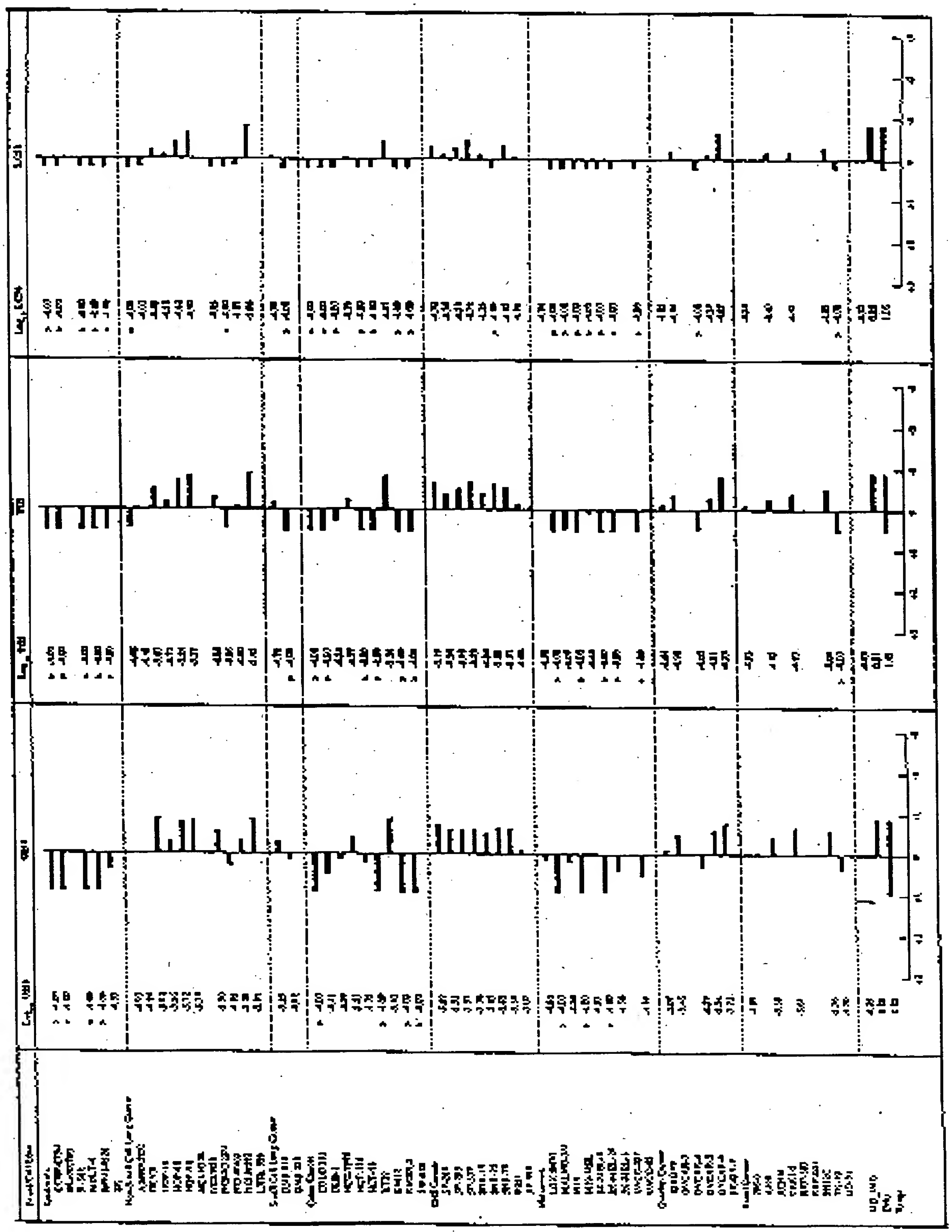


Fig. 1

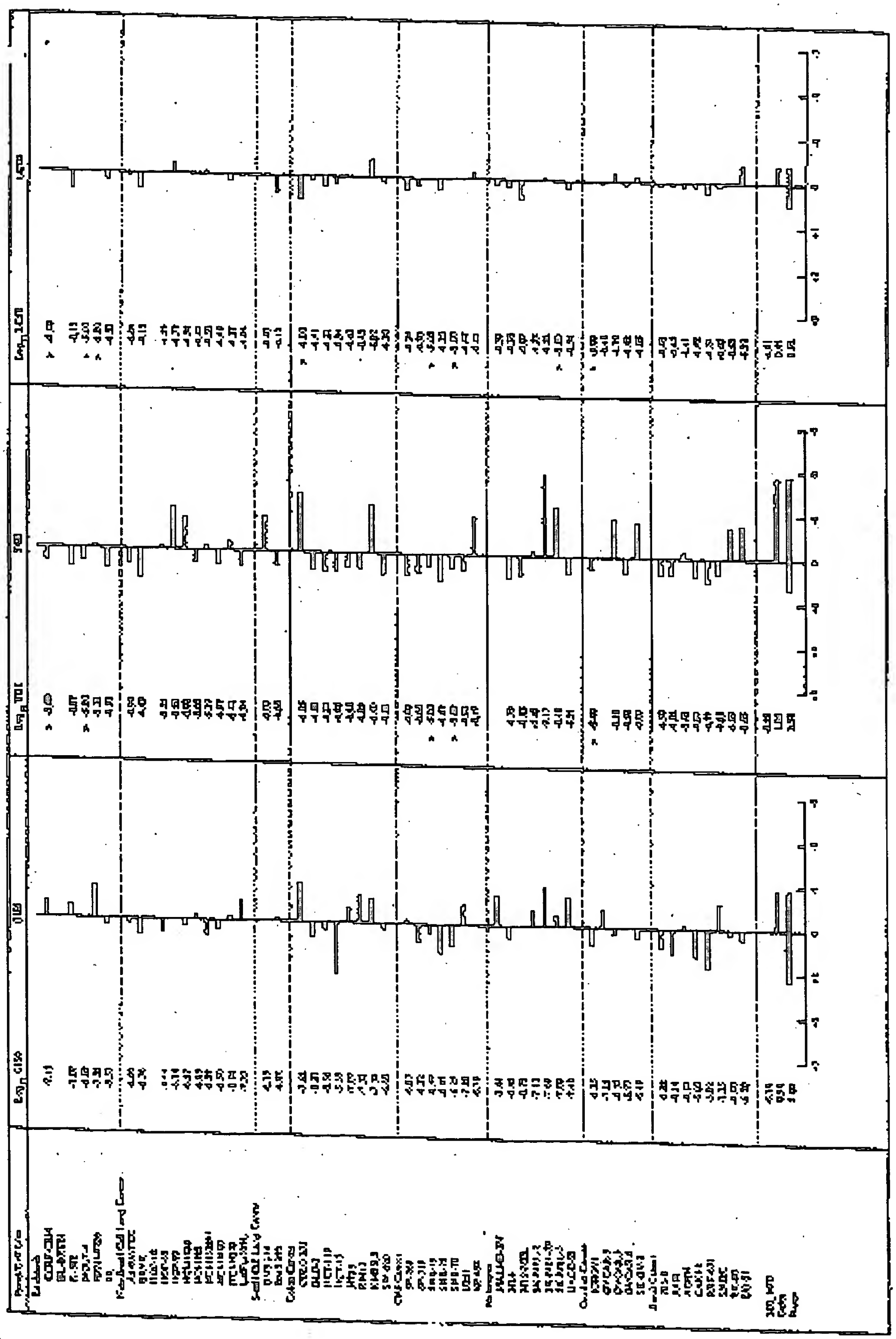


Fig. 2

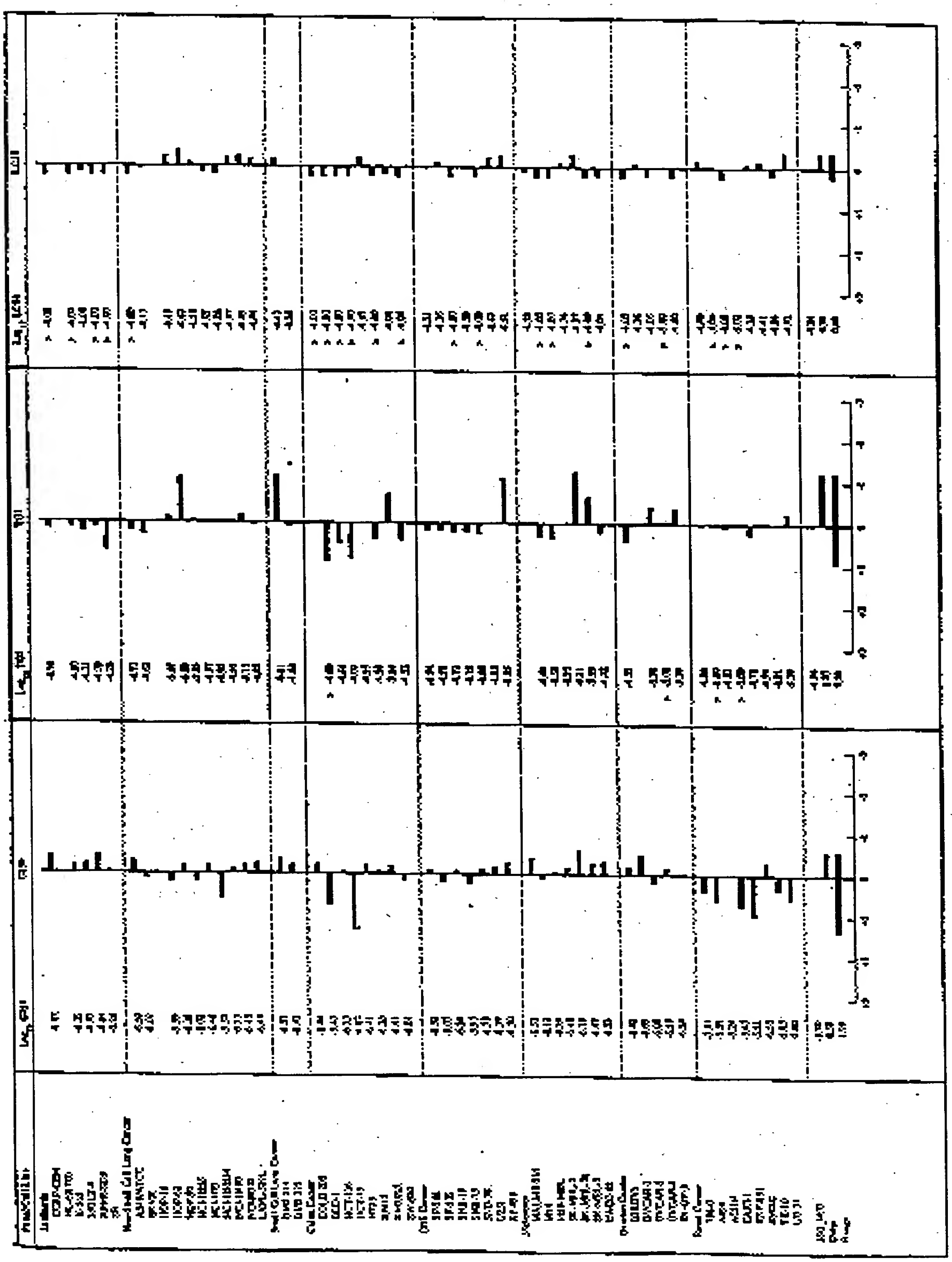


Fig. 3

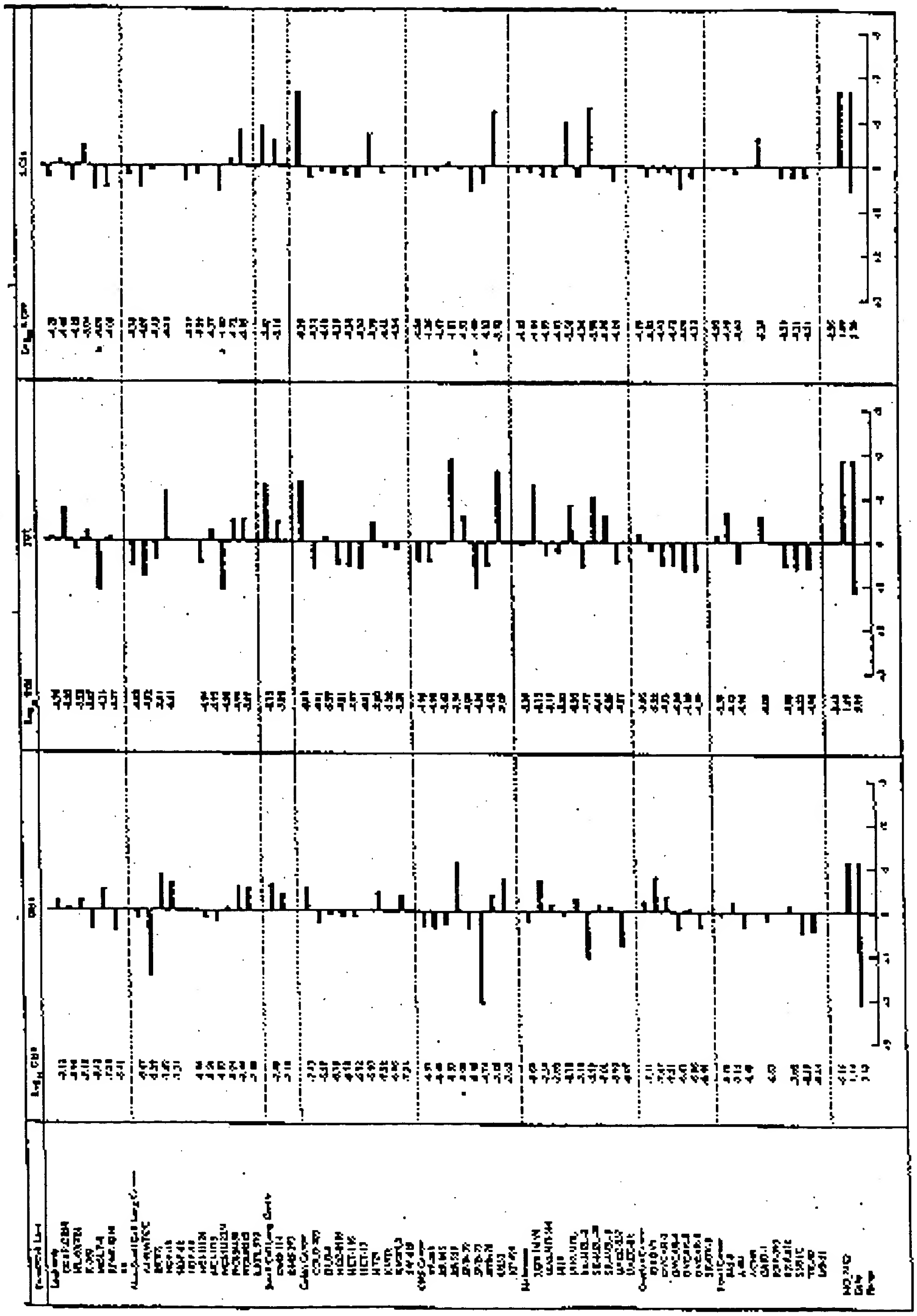


Fig. 4.

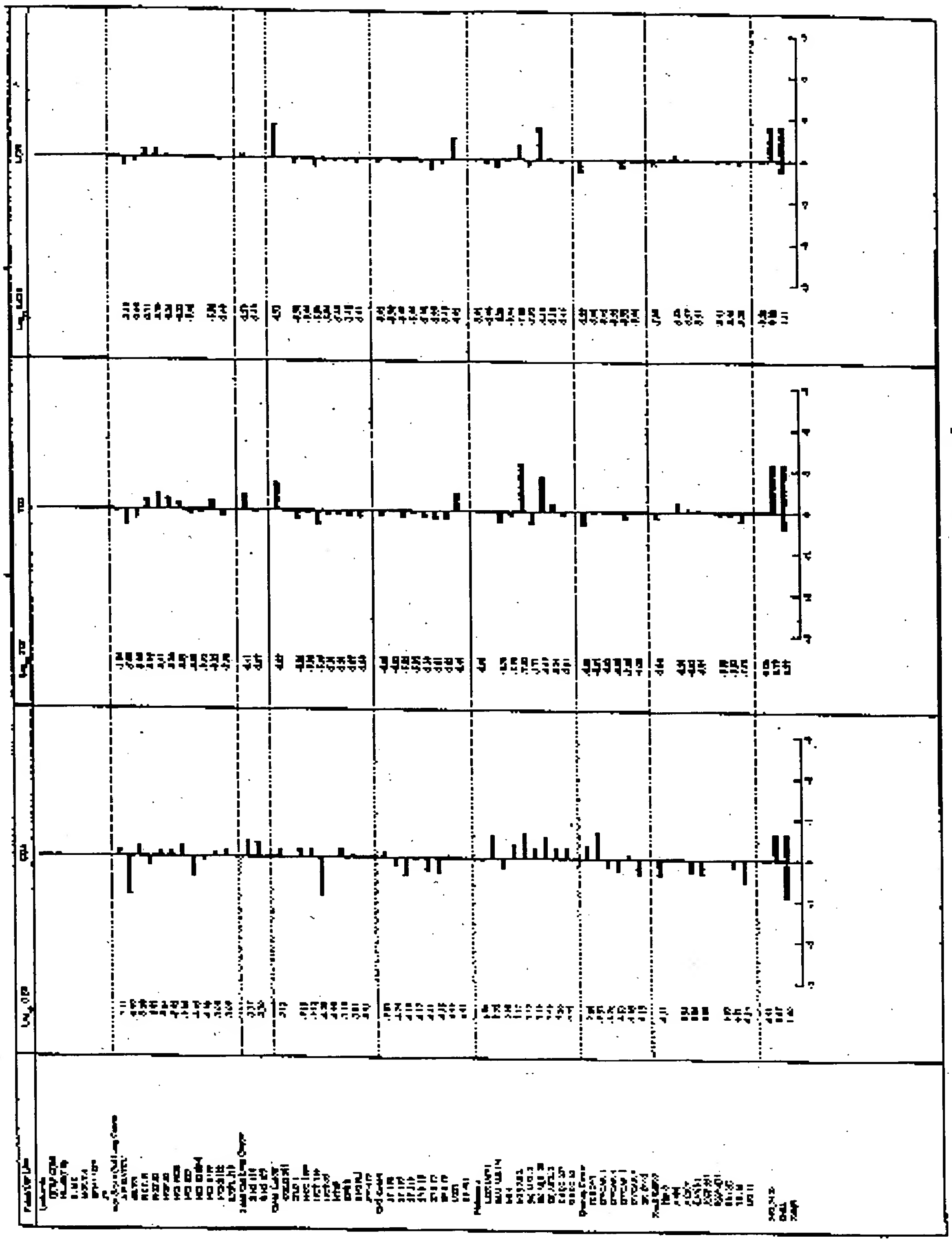


Fig. 5.

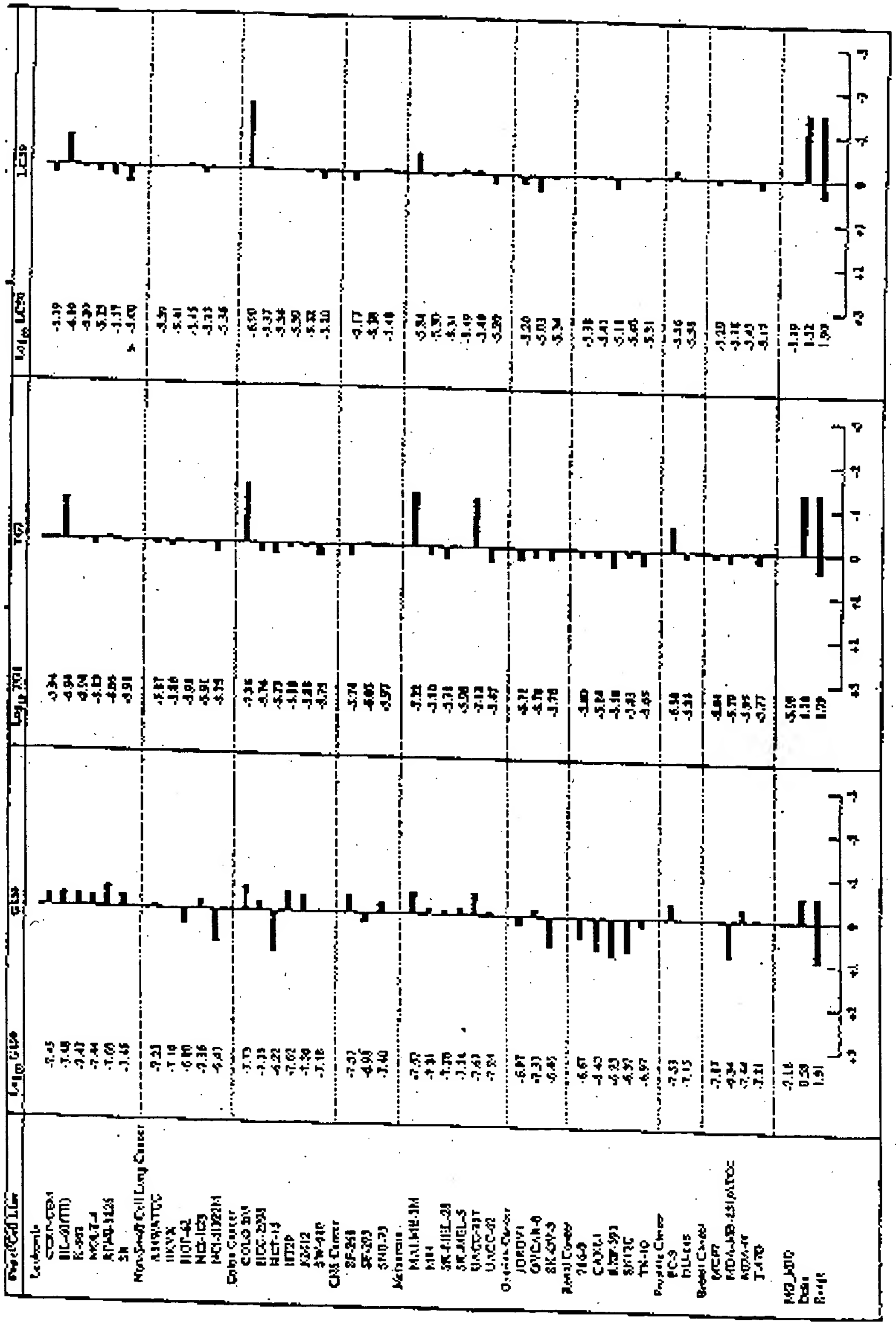


Fig. 6

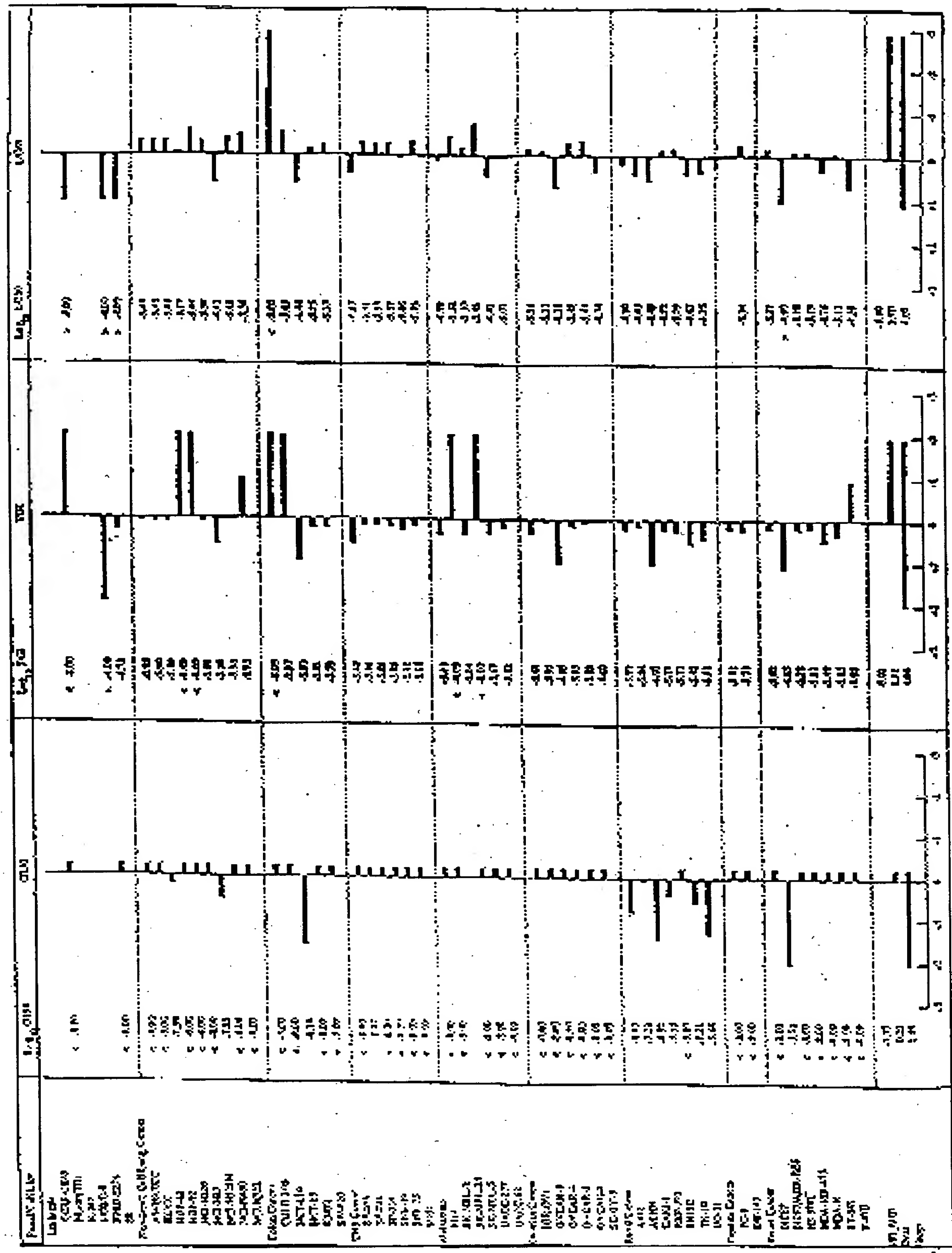


Fig. 7.